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# Chronic Cassava Toxicity

Proceedings of an interdisciplinary workshop
London, England, 29-30 January 1973

**Editors: Barry Nestel and Reginald MacIntyre**

INTERNATIONAL DEVELOPMENT RESEARCH CENTRE
CENTRE DE RECHERCHES POUR LE DÉVELOPPEMENT INTERNATIONAL

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1973

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*Editors*: BARRY NESTEL AND REGINALD MACINTYRE

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*"...cassava...provides a major source of calories to, perhaps, 300 million people..."* (from the Foreword)

# Foreword

Although cassava (*Manihot esculenta* Crantz) is one of the world's most important staple food crops and provides a major source of calories to, perhaps, 300 million people, it is a crop that has, until recently, been relatively neglected by research workers. There appear to be several reasons for this, in particular the fact that cassava is traditionally a crop used by low-income people in the tropics. It is bulky, high in energy, and low in protein and not particularly attractive to western palates. Because it does not tolerate low temperatures, it grows only in the tropics. It normally deteriorates rather rapidly after harvesting and is a crop with which most people in temperate regions are not familiar.

For a variety of reasons cassava is a difficult crop to work with from the agronomic and plant-breeding standpoints, and it has been virtually ignored by tropical research workers who have preferred to concentrate their activities on export crops.

In the past five years, two major new international agriculture research centres have been created which include cassava amongst the commodities which they are studying. At the International Centre for Tropical Agriculture (CIAT) in Colombia, cassava is a main program activity and a team of well-financed international scientists are concentrating their activities on this commodity. At the International Institute for Tropical Agriculture (IITA) in Nigeria, a program of somewhat similar magnitude is being mounted to study both cassava and yams. The formulation and structuring of these two new international programs, which are devoting far more resources to cassava than have been available previously, have posed a number of important questions on research policy.

One such question concerns the importance of research on the cyanogenic glucosides of cassava. These have been known to be responsible for both acute and chronic toxicity in humans for some time. However, the importance of chronic toxicity in man has been studied extensively only in the last decade. Recent work has indicated not only that high cassava intakes are associated with the incidence of tropical ataxic neuropathy in Africa, but that in the presence of marginal iodine and low protein intakes, high cassava diets may be a permissive factor in the development of goitre and cretinism. In certain parts of Africa where cassava intakes are high ataxic neuropathy or disturbances in thyroid metabolism have assumed endemic proportions.

Domestic livestock is seldom fed high levels of cassava for prolonged periods but nevertheless there is evidence to indicate that impaired growth occurs in pigs and poultry on high cassava diets unless these are supplemented with methionine. Since cassava is assuming an increasing importance as an animal feed, the question of its possible toxicity has aroused some interest. The international trade in dried cassava products used for animal feed is now valued at about $70 million a year and is projected to triple within the next decade.

Because of the International Development Research Centre's heavy commitment in funding cassava research, and because of the many unknowns associated with the toxicity problem in both humans and animals, it was considered desirable to call together a group of people who are interested in the cyanogenic glucosides and in cassava toxicity, to review the state of knowledge in these fields within the context of current research programs on cassava.

A two-day interdisciplinary workshop was held in London, England, on 29–30 January 1973.

The first four papers at the workshop set the framework against which the toxicity discussions took place. The introductory paper by *Nestel* presented an overview of the role cassava plays in the world of today and went on to explore the future potential for this crop. It was suggested that the use of cassava as both human and animal feed was likely to increase in the immediate future. *Coursey* then discussed the nature of cassava toxicity and the traditional technology adopted by cassava-eating communities in order to reduce toxic levels. Following these background papers on past, present, and future utilization prospects, *Cock* from CIAT in Colombia and *Sadik* from IITA in Nigeria described the ongoing cassava research programs of their institutes, particularly from the standpoint of their interests in cassava toxicity.

The cyanogenic character of cassava was reviewed by *de Bruijn* who referred to the extensive study on the agronomic and physiological aspects of cassava toxicity which he had carried out in the Ivory Coast. The genetics of cyanogenesis in cassava does not appear to have been studied but an interesting contribution from *Hughes* examined this process in other plant species and suggested that genetic manipulation might offer a mechanism for reducing toxicity in cassava.

The biosynthesis of cyanogenic glucosides was reviewed in a series of three closely interwoven papers. *Conn* introduced the subject by discussing the occurrence, biosynthesis, and functions of these glucosides prior to *Butler* describing the physiological and genetic aspects in cassava and other plants. The discussion then focussed very specifically on cassava with a paper by *Nartey* which examined the biosynthesis of linamarin and lotaustralin in cassava.

The improvements in analytical techniques in recent years and technical difficulties associated with adequately sampling cassava have thrown some doubt on the validity of some of the earlier experimental data on glucoside and enzyme levels in cassava tissues. The techniques and problems of adequate assay were reviewed by *Zitnak* whose presentation was followed by a review by *Oke* who dealt with the various modes of cyanide detoxification. Yet another review, this time by *Hill*, examined the subject of chronic cyanide toxicity in domestic animals. This subject was expanded on by *Maner* and *Gomez* who outlined the results from some of their very extensive series of animal-feeding studies using high cassava rations.

A series of five papers discussed the theme of cyanide toxicity in humans. *Wilson* explored this from the standpoint of cyanide–vitamin $B_{12}$ relationships. *Osuntokun*, in a wide ranging review of the subject, identified chronic cyanide intoxication due to dietary cassava derivatives as being the major etiological factor in the development of tropical ataxic neuropathy in Nigeria and possibly other parts of Africa. Although there was a slightly higher incidence of goitre in Osuntokun's patients it was much less common than in the high cassava-eating areas in Eastern Nigeria and in Zaïre reported on by *Ekpechi* and by Delange, Ermans, and their co-workers. *Delange et al.* showed that the antithyroid action of cassava was related to the endogenous production of thiocyanate from cyanide released by the cyanogenic glucosides present in cassava and that in the presence of an iodine deficiency cassava constituted a goitrogenic factor. *Ermans et al.* concluded the opening day's presentation by reporting on the mechanism of the goitrogenic action of cassava.

On the second day of the meeting, the emphasis was given over to three interdisciplinary theme discussions. In the first of these, a discussion on the biosynthesis of cyanogenic glucosides in cassava was introduced by *Conn*. The session covered the fields of chemistry, biosynthesis, plant physiology, the site of synthesis, translocation, and genetics.

Following this *Fowden* chaired a review of the metabolic pathways of cyanogenic glucosides in the human and animal body. This covered the aspect of cyanogenesis, the toxicity of glucosides, and the distribution of $\beta$-glucosidases, cyanide metabolism, species

differences, iodine metabolism, and an examination of the protective versus the attractive roles of cyanides in the tissues.

The session on reducing cassava toxicity which was led by *Coursey* discussed the potential for reducing the toxicity through plant breeding, food technology, and nutritional habits.

A synthesis of the conclusions of each of the three theme sessions was presented to the final meeting chaired by *Wilson*. Attention was also given to the subject of the choice of species for animal experimentation and to the need for a supply of purified linamarin for further investigational work.

In these proceedings, the 18 working papers are presented in full and are followed by a short summary of the theme discussions which is based upon working notes provided by session chairmen and rapporteurs. The range of disciplines represented at the workshop, and the number of papers discussed, led to the identification of a number of areas in which more research appears to be needed.

This workshop is one in a series that IDRC has sponsored in conjunction with CIAT and IITA with the objective of defining cassava program priorities. We are deeply indebted to the participants for the time and effort they devoted to preparing and presenting material for this meeting.

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# Current Utilization and Future Potential for Cassava

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**Abstract** Current annual world production of cassava totals over 90 million tons, most of which is consumed by people living close to subsistence levels in tropical areas. However, there is a long-standing trade in cassava starch. It has viscosity and tensile characteristics of particular value for certain processes in the textile, paper, and food industries. In the last decade a growing trade has developed in the use of dried cassava chips or pellets as an animal feed component. This trade is principally between Indonesia and Thailand on one hand, and Belgium, Holland, and West Germany on the other, and is worth about U.S. $70 million a year to the exporting countries.

The production costs of cassava under current systems of husbandry are such that it appears to be highly competitive in cost terms with other types of energy foods grown in the lowland tropics. Because of the limited alternatives in many cassava-producing areas, the ease of growing the crop, and the demographic trends, it is expected that food use will grow at 2.5–3% per annum and that by the end of this century the number of people deriving a major calorie intake from cassava (currently between 200 and 300 million) will have doubled.

Because of the complexity and competitiveness of the international starch market, growth prospects in this sector are difficult to predict. However, in the animal feed sector the growth potential appears very promising. The expanded EEC is projected to absorb three times its current level of 1.5 million tons of cassava (equivalent to 5 million tons of fresh roots) by 1980, providing tariff changes and production costs do not change significantly. In the cassava-producing countries themselves both feed grains and animal proteins are generally in short supply and there appear to be very good prospects for developing cassava-based compound livestock feeds. These prospects are heightened by the recent initiation, for the first time, of a well-financed major international research effort to study cassava from the standpoint of increasing its yield, improving its quality, and lowering production costs.

Bearing in mind the fact that chronic toxicity in humans and animals on high cassava diets is already a well-recognized problem it would appear that unless effective steps can be taken to reduce this toxicity, it could become a problem of increasing significance both from the nutritional standpoint and in retarding both domestic and export earnings in producing countries.

**Résumé** La production mondiale du manioc dépasse présentement 90 millions de tonnes, consommée en grande partie par les populations des régions tropicales dont le niveau de vie est à peine au-dessus de celui de subsistance. Par ailleurs, l'amidon du manioc est depuis longtemps l'objet d'un commerce important. Sa viscosité et son élasticité lui donnent une valeur particulière pour la fabrication des textiles, du papier et des produits alimentaires. La dernière décennie a vu naître un commerce qui se développe rapidement et qui utilise le manioc séché, en flocons ou en boulettes, dans la fabrication des moulées pour le bétail. Ce commerce se pratique surtout entre l'Indochine et la Thaïlande d'une part, et la Belgique, la Hollande et l'Allemagne de l'Ouest d'autre

part. Il représente une somme d'environ 70 millions de dollars (E.-U.) par année pour les pays exportateurs.

Les pratiques agricoles courantes assurent au manioc des coûts de production qui semblent hautement compétitifs avec les autres types d'aliments énergétiques cultivés dans les terres basses tropicales. A cause des possibilités restreintes de cultures alternatives dans les régions productrices du manioc, de la facilité de sa culture et des tendances démographiques actuelles, on s'attend à une augmentation de la consommation du manioc au rythme de 2.5 à 3% par année. On prévoit également qu'à la fin du siècle, le nombre de personnes pour lesquelles le manioc est la principale source de calories (200–300 millions présentement) aura doublé.

Par suite de la complexité et de la concurrence du marché international de l'amidon, le potentiel de croissance dans ce secteur est difficile à prédire. Par contre, le secteur des moulées pour le bétail semble offrir d'intéressantes perspectives de développement. Il est à prévoir qu'une CEE élargie aura triplé en 1980 ses besoins présents de 1.5 millions de tonnes du manioc (équivalent à 5 millions de tonnes de racines fraîches), pourvu que les échanges tarifères et les coûts de production demeurent sensiblement les mêmes. Dans les pays producteurs du manioc, les apports de grains de moulées et de protéines animales ne peuvent généralement suffire à la demande. Il semble donc y avoir d'excellentes perspectives pour les moulées à base du manioc. Ces perspectives sont réhaussées par la mise en marche, récemment et pour la première fois, d'un programme de recherche à l'échelle internationale et à fonds suffisants, visant à améliorer le rendement et la qualité et à minimiser les coûts de production du manioc.

La toxicité chronique produite chez l'homme et le bétail par un régime riche en manioc est un problème reconnu. Si on ne prend pas de mesures efficaces pour réduire cette toxicité, le problème deviendra de plus en plus sérieux, tant au point de vue de l'alimentation qu'au point de vue de la limitation des bénéfices, sur les marchés domestiques aussi bien qu'à l'exportation, des pays producteurs.

THE purpose of this introductory paper is to present an overview of the role that cassava plays in the world today and then to explore the future potential for this commodity. Some of the data and ideas brought out in the paper will suggest that the use of cassava as both human and animal food is likely to increase in future years. Bearing in mind the fact that chronic toxicity in humans and animals on high cassava diets is already a well-recognized problem, it would appear that unless effective steps can be taken to reduce this toxicity it could become a problem of increasing significance in the future. Apart from the nutritional implications of this, it appears possible that toxicity could also play an important role economically in, perhaps, retarding the development of new markets for cassava both domestically and overseas.

## Production

Although production statistics for cassava are notoriously unreliable, the best available evidence indicates that, on a tonnage basis, annual world production is only exceeded by that of six other crops (Table 1).

Cassava is produced in more than 80 countries, but two thirds of world production takes place in only five of them (Brazil, Indonesia, Zaïre, Nigeria, and India) and 90% of global production comes from 19 countries (Table 2; Fig. 1).

In producing countries, production has grown steadily at about the same rate as the population increase during the past 20 years. Most of the increase in production appears to be due to an increased area under the crop since only limited changes in yield have been reported. There is, however, a wide range of variation in yields, with a global average of 9.4 tons/ha. A number of countries, especially in Africa, have average yields of less than half this figure whereas others report averages exceeding 20 tons/ha.

Under experimental conditions, yields of more than 70 tons/ha have been taken in a 12-month period in spite of the fact that cassava has, as yet, received relatively little attention from agricultural scientists. Although there are major difficulties in bringing about yield and quality improvements in this species, the potential at least for yield improvements does appear to be considerably greater than that of many plant species that have been subjected to intensive study for many years.

TABLE 1. World production, acreage, and yield of selected crops, 1971 (source: FAO Production Yearbook 1971).

| | World hectarage *(million ha)* | World yield *(100 kg/ha)* | World production *(million metric tons)* |
|---|---|---|---|
| *Cereal grains* | | | |
| Wheat | 217.2 | 15.8 | 343.1 |
| Rice (paddy) | 134.9 | 22.8 | 307.4 |
| Maize | 112.9 | 27.3 | 307.8 |
| Millet and sorghum | 113.4 | 8.9 | 101.1 |
| Barley | 82.2 | 18.5 | 152.7 |
| Oats | 31.2 | 18.5 | 57.7 |
| Rye | 19.7 | 15.7 | 30.9 |
| *Root crops* | | | |
| Potatoes | 22.5 | 136.0 | 306.4 |
| Sweet potatoes and yams[a] | 17.0 | 87.0 | 147.7 |
| Cassava[a] | 9.8 | 94.0 | 92.2 |
| Sugar beets | 7.6 | 29.9 | 228.2 |
| *Legumes (pulses)* | | | |
| Soybeans | 36.2 | 13.3 | 48.3 |
| Pigeon peas | 2.9 | 6.8 | 2.0 |
| Dry beans | 22.9 | 5.1 | 11.7 |
| Peanuts | 18.8 | 9.8 | 18.5 |
| Chick-peas | 10.2 | 6.6 | 6.7 |
| Cowpeas | 3.1 | 3.7 | 1.1 |
| Dry broad beans | 4.7 | 11.2 | 5.2 |
| Dry peas | 9.0 | 12.2 | 10.9 |

[a]1970 data.

Cassava is generally grown as a subsistence crop. It is particularly valued because of its drought tolerance, its ability to grow in poor soils, and its relative resistance to weeds and insect pests. These characteristics, plus the fact that it can be left in the ground without harvesting for a lengthy period of time, mean that it is a very useful crop as a security against famine. Furthermore, it is not season-bound and can, therefore, be planted and harvested at any period of the year. For these reasons cassava is obviously an attractive crop for the subsistence farmer for whom risk aversion must, of necessity, be an important value objective. Indeed for such a farmer, the security of being able to harvest a crop in adverse times may be more important than the desire to harvest a higher yield, although as development brings subsistence producers into a market economy the production of marketable surpluses can be expected to assume increasing importance.

Cassava also possesses certain characteristics which make it of particular interest to the biologist and to the economist concerned with resource development in tropical areas. First and foremost of these is the fact that cassava productivity in terms of calories per unit land area per unit of time appears to be significantly higher than that of other staple food crops (de Vries et al. 1967).

Coursey and Haynes (1970) indicated that cassava can produce $250 \times 10^3$ cal/ha per day as compared to $176 \times 10^3$ for rice, $110 \times 10^3$ for wheat, $200 \times 10^3$ for maize, and $114 \times 10^3$ for sorghum. They also point out that the grain crops have been subjected to considerable research to improve their genetic potential, whereas cassava offers considerable scope for genetic improvement. Coursey and Haynes also indicate that root crops have a higher biological efficiency as food producers. They attribute this efficiency to structural engineering considerations since the edible part of tuberous roots lies beneath the ground and does not have to be supported by a stem. In fact 60–85% of the total dry weight of root crops may be edible whereas in wheat the figure is only up to 36%.

TABLE 2. World production of cassava in 1970 (source: FAO Production Yearbook 1971).

| | Million tons | % world production |
|---|---|---|
| Brazil | 29.5 | 32.6 |
| Indonesia | 10.5 | 11.4 |
| Zaïre | 10.0 | 10.9 |
| Nigeria | 7.3 | 7.9 |
| India | 5.2 | 5.6 |
| | | 67.9 |
| Mozambique | 2.1 | 2.4 |
| Uganda | 2.0 | 2.2 |
| Thailand | 2.0 | 2.2 |
| Paraguay | 1.8 | 2.0 |
| Burundi | 1.6 | 1.7 |
| Ghana | 1.6 | 1.7 |
| Angola | 1.6 | 1.7 |
| Tanzania | 1.5 | 1.6 |
| Madagascar | 1.2 | 1.3 |
| Togo | 1.2 | 1.3 |
| Colombia | 1.2 | 1.3 |
| Central African Republic | 1.0 | 1.1 |
| | | 20.4 |
| Cameroon | 0.9 | 1.0 |
| Dahomey | 0.7 | 0.8 |
| North Vietnam | 0.7 | 0.8 |
| Ivory Coast | 0.5 | 0.6 |
| Guinea | 0.5 | 0.6 |
| Peru | 0.5 | 0.6 |
| | | 4.1 |
| 63 Other Countries | 7.1 | 7.7 |
| Total: | 92.2 | 100.0 |

In order to utilize the biological efficiency of cassava and to develop the crop, uses for it have to be found. Furthermore, it has to be produced at a price at which these uses are economic. Utilisation prospects appear to vary widely between different countries. Thus the Thai farmer who grows most of the cassava which reaches the world market obtains U.S. $11–12 for the farm-dried chips from a ton of fresh cassava whereas for fresh cassava for human use, the Jamaican farmer obtains 2–4 times this price (Rankine and Houng 1971) and, at certain times of year, the Colombian farmer may obtain 6–10 times the Thai price (P. Pinstrup-Andersen, CIAT, personal communication). However, generally speaking the farm price seems to lie in the range of U.S. $10–15/ton of fresh root equivalent.

It is difficult to cost cassava production since the main inputs are family labour and land, and in subsistence-farming areas, the land is often communally owned. Brannen (1972) reviewed some of the literature on production costs and found that the usual cost of producing cassava was about U.S. $6/ton. The major production cost was labour. For a variety of reasons, it is difficult to compare the various labour costings available, but in various surveys the man-hours used to produce a ton of cassava appeared to range from 50 to 200 and to average about 100 (Brannen 1972; Rankine and Houng 1971; Raeburn et al. 1950). Obviously the return to labour from cassava production is very low, notwithstanding the fact that Raeburn et al. found the yield per man-day from cassava production exceeded that of other tropical staples. Clarke and Haswell (1964) reported a similar finding when comparing both the output value and the labour productivity of various tropical crops in terms of FAO standard wheat equivalents.

The low return to labour relates to the fact that the opportunity cost of labour in many subsistence areas is often regarded as being close to zero, otherwise a production cost per ton of U.S. $6 would not be possible. However we may anticipate that some mechanisation may be necessary in the future since, as economies develop, labour generally tends to demand a higher return, especially for an unpleasant job such as harvesting cassava (which commonly accounts for 25–30% of the total labour costs). For this reason production costs may be expected to rise. Against this it must be taken into account that subsistence yields are often only about 10% of the production potential of the crop, so that there is a great deal of scope for reducing the costs of production by raising yields. This is especially true if high-yield varieties can be produced that are more easily harvested (by either man or machine) but still retain adequate drought and disease tolerance. In view of the limited past resources devoted to both the breeding and the mechanisation of cassava, compared with those now available, it is difficult to make any forecasts of the likely future pattern of production costs. What does appear probable is that these costs will be strongly influenced by the results of plant-breeding work in terms of the achievements that are made with respect to yield and morphology (and perhaps cyanogenic glucoside content).

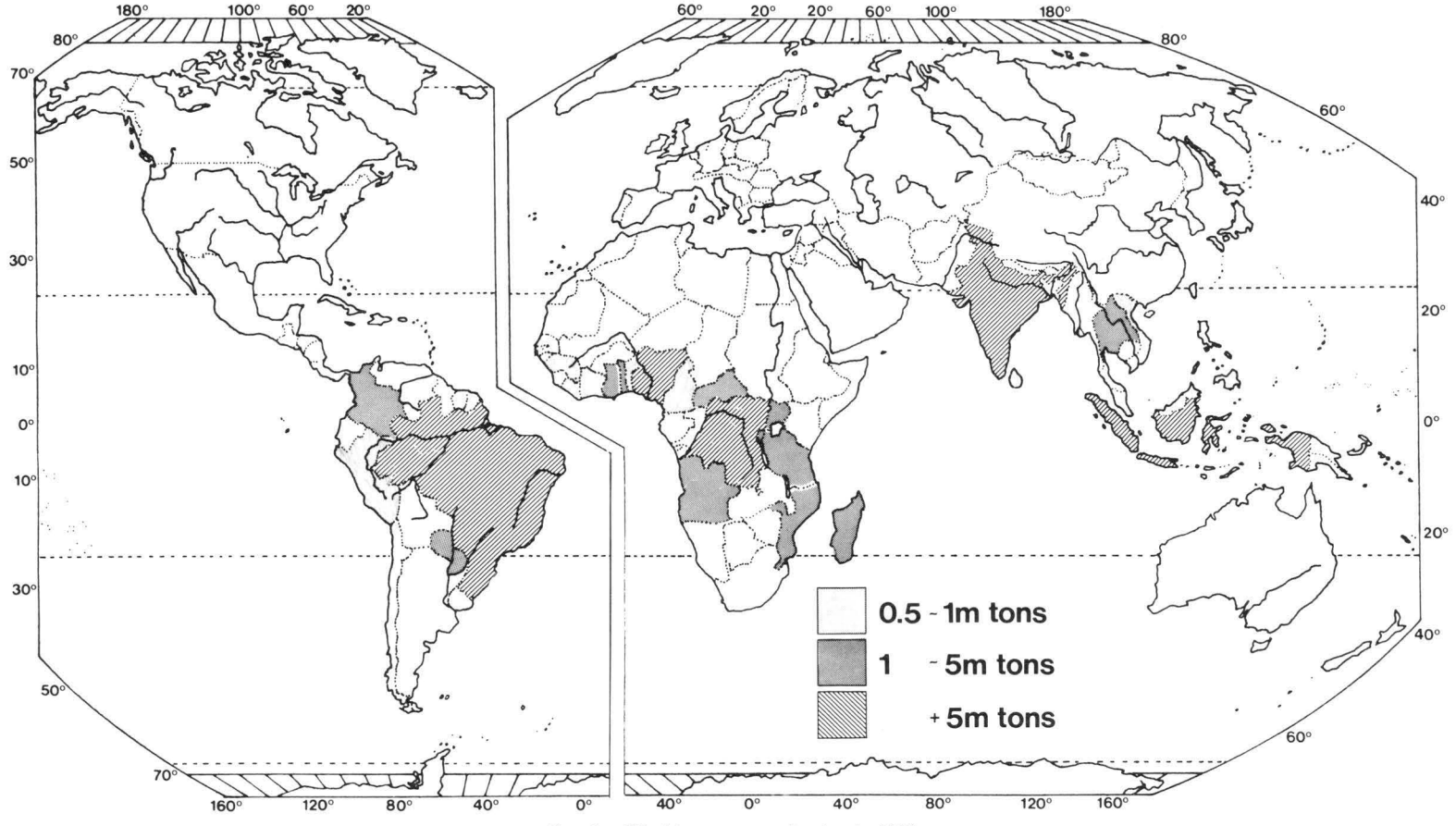


FIG. 1. World cassava production in 1970.

## Consumption

The most important use of cassava is as human food (Fig. 2). Consumption data are presented in Table 3 from 14 countries in which cassava is an important dietary staple. These national data may be misleading in some of the larger countries in which cassava is the primary staple in certain areas but not in others. For example, cassava is more important in the south and east of Nigeria than in the north. In a survey of southern Nigeria, Nicol (1952) found that 25–56% of the dietary calories came from cassava (as opposed to the national figure of 14% in Table 3). Bailey's (1961) surveys in Java showed 63.5% of the calorie intake (as opposed to the Indonesian national figure of 15.2%) came from cassava. Normanha (1970) reported that in Brazil in 1962–63 cassava consumption was 124 kg/person per annum at the national level. This represented an urban intake of 42 kg and 200 kg in rural areas. By comparison, it is interesting to note that the average intake of wheat (in the form of

TABLE 3. Human intake of cassava in 14 countries 1964–66 (source: FAO Food Balance Sheets 1964–66).

| | Human population *(million)* | Cassava as % total caloric intake | Cal/day from cassava | Cassava per year *(kg)* |
|---|---|---|---|---|
| Congo (Brazzaville) | 0.84 | 54.8 | 1184 | 470 |
| Zaïre | 15.63 | 58.5 | 1193 | 437 |
| Central African Rep. | 1.33 | 48.7 | 1057 | 354 |
| Gabon | 0.46 | 47.0 | 1027 | 342 |
| Mozambique | 6.96 | 42.6 | 908 | 304 |
| Angola | 5.15 | 34.5 | 659 | 220 |
| Liberia | 1.08 | 26.2 | 600 | 201 |
| Togo | 1.64 | 26.5 | 590 | 197 |
| Dahomey | 2.36 | 20.1 | 438 | 148 |
| Paraguay | 2.03 | 19.7 | 540 | 181 |
| Ghana | 8.14 | 18.2 | 380 | 130 |
| Brazil | 80.77 | 10.8 | 274 | 107 |
| Nigeria | 58.48 | 14.1 | 306 | 103 |
| Indonesia | 105.74 | 15.3 | 269 | 92 |
| Total: | 304.15 | – | – | – |
| Weighted avg. (14 countries) | – | 19.4 | 374 | 124 |

TABLE 4. Daily purchases expressed in calories, and price per 1000 calories of selected staple feeds, Kumasi and Sekondi-Takoradi, Ghana, 1955 (Johnston and Kaneda 1960).

| | Kumasi | | Sekondi-Takoradi | |
|---|---|---|---|---|
| | Purchases *(cal/person/day)* | Price *(¢/1000 cal)* | Purchases *(cal/person/day)* | Price *(¢/1000 cal)* |
| Cassava and products | | | | |
| Fresh roots | 243 | 2.68 | 456 | 2.73 |
| Gari (meal) | 46 | 2.94 | 64 | 3.23 |
| Kekonte (dried roots) | 212 | 1.63 | 57 | 2.69 |
| Plantains | 389 | 3.05 | 168 | 4.32 |
| Yams | 123 | 5.91 | 49 | 7.64 |
| Maize and products | | | | |
| Kenkey | 50 | 5.74 | 188 | 5.33 |
| Dough | 43 | – | 49 | – |
| Rice | 101 | 5.20 | 111 | 5.28 |
| Cocoyams | 98 | 3.76 | 15 | 5.02 |
| Bread | 27 | 11.03 | 47 | 11.70 |
| All starchy staples | 1364 | – | 1260 | – |

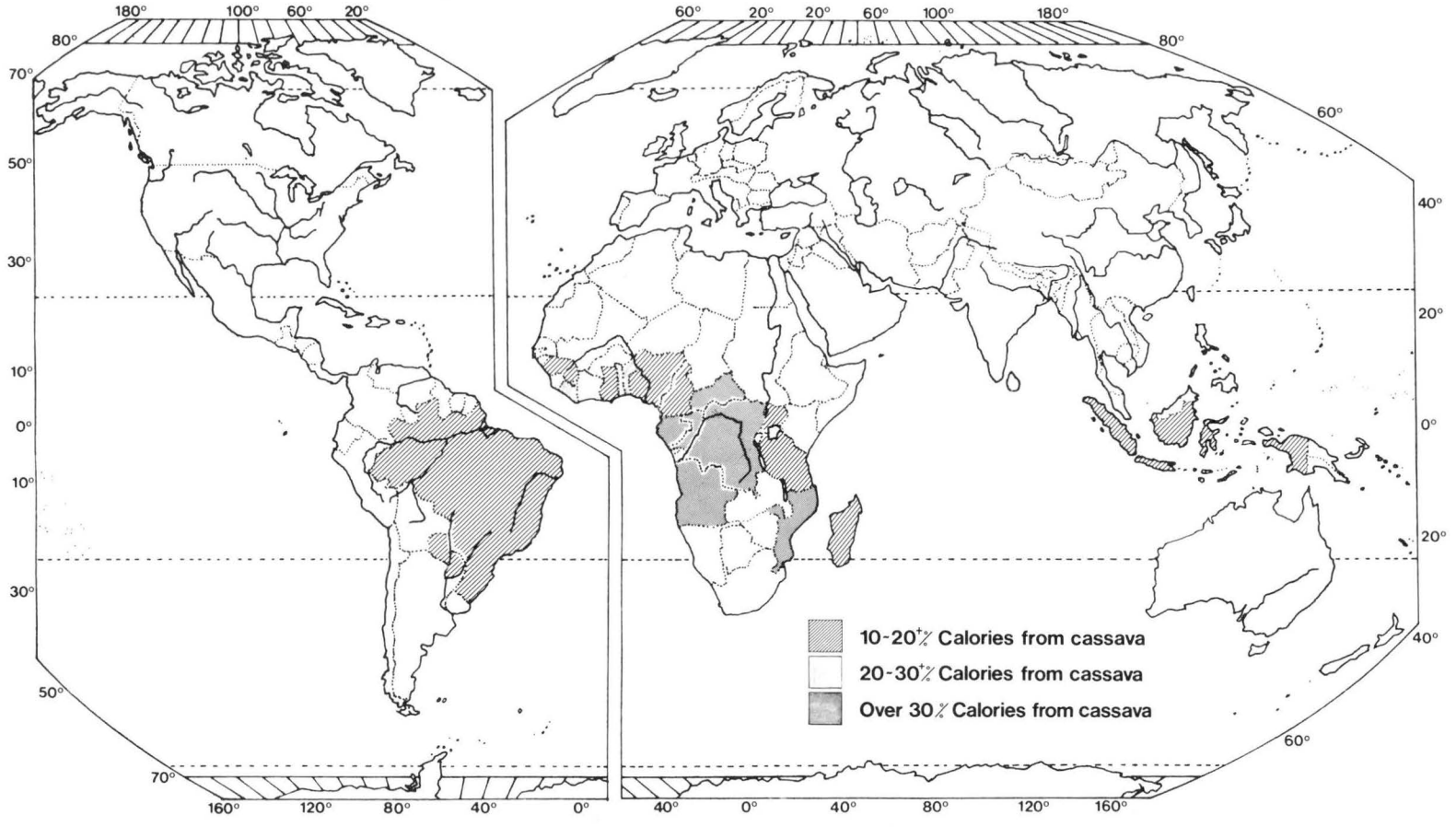


FIG. 2. Cassava consumption levels in 20 countries in 1964–66.

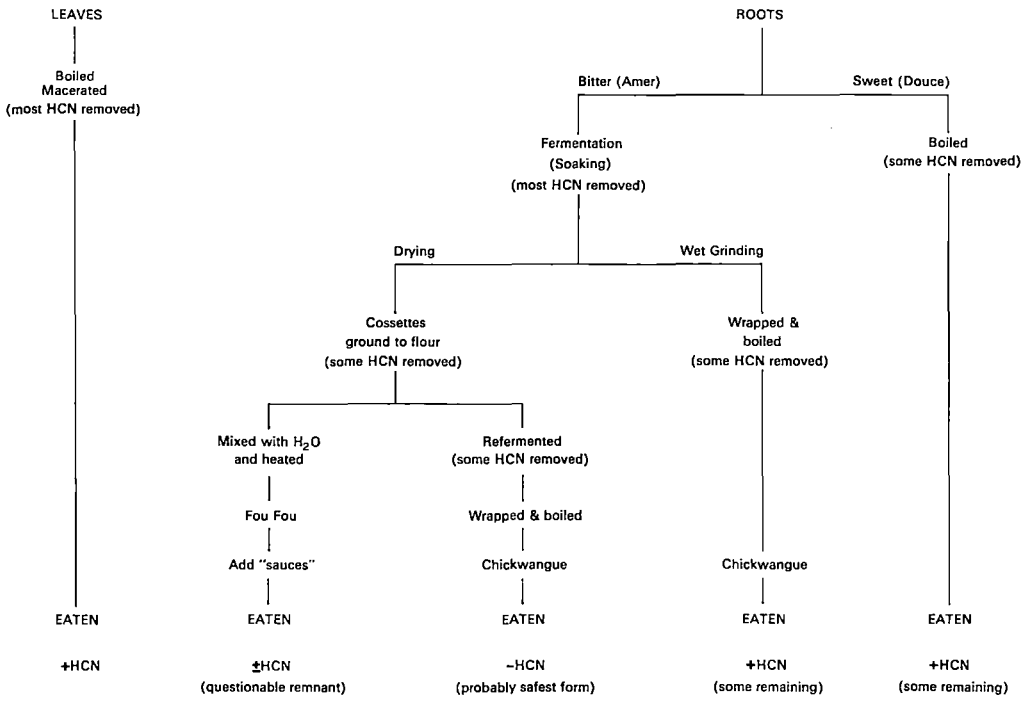


FIG. 3. Cassava use in the Kinshasa area, Zaïre (source: Rogers et al. 1971).

flour) in west European and North American countries is 50–80 kg/person per year, providing between 500 and 800 cal/day.

Wheat, of course, does tend to be the preferred energy staple in many non rice-growing parts of the world but the difficulty of growing it in the tropics usually makes it an expensive item. Bearing in mind the low income normally encountered in tropical areas (where annual per capita incomes rarely exceed $200 and are often under $100), the relative cost of cassava and its products is of considerable significance. Table 4 presents data from two areas of Ghana where calorie intakes from cassava averaged around 500/day at a daily cost of under U.S. 2¢. This contrasts rather favourably in cost terms with calories derived from other staples.

Table 4 also indicates that cassava is marketed in Ghana in various forms. This is a common situation in many cassava-producing areas. The marketing system is often fairly complex with a large variety of cassava products being handled. Figure 3 shows the variety of forms in which cassava is consumed in one part of Zaïre. The range of processed products and the number of stages in marketing both tend to depress the producers' share of the price of the product that is finally consumed.

In areas where cassava intakes are high there are sometimes problems by virtue of the low content of essential amino acids in the root (Table 5). The essential amino acid profile of cassava also indicates that it is particularly deficient in sulfur-containing amino acids (Bailey 1961). The significance of this in the detoxification of cyanogenic glucosides is discussed in several papers in these proceedings.

## Industrial Use

As already noted, cassava is used to make a large number of processed products, most of which involve some form of drying and/or fermentation. One of the most important of these products is industrial starch. Cassava starch contains only 17% amylose as opposed to 22% in potato starch and 27% in corn starch and because of this it possesses unusual viscosity characteristics. The large percentage of branch-chained amylopectins

TABLE 5. Amino acid content/100 g food (source: FAO Amino Acid Content of Foods and Biological Data on Proteins. Nutritional Studies No. 24, Rome 1970).

| Food | Moisture (g) | Nitrogen (g) | Conversion factor (W) | Protein (g) | Lysine (mg) | Methionine (mg) | Threonine (mg) | Tryptophan (mg) | Total essential amino acids (mg) | Total amino acids (mg) |
|---|---|---|---|---|---|---|---|---|---|---|
| *Cereal grains* | | | | | | | | | | |
| Barley | 12.0 | 1.88 | 5.83 | 11.0 | 406 | 196 | 389 | 180 | 4,203 | 11,118 |
| Maize | 12.0 | 1.52 | 6.25 | 9.5 | 254 | 182 | 342 | 67 | 3,820 | 9,262 |
| Millet | 11.0 | 1.55 | 6.25 | 9.7 | 332 | 239 | 374 | 189 | 3,979 | 9,505 |
| Oats | 10.0 | 2.23 | 5.83 | 13.0 | 517 | 234 | 462 | 176 | 5,169 | 12,998 |
| Rice (brown) | 13.0 | 1.26 | 5.95 | 7.5 | 299 | 183 | 307 | 98 | 3,033 | 7,973 |
| Rice (polished) | 13.0 | 1.13 | 5.95 | 6.7 | 255 | 150 | 234 | 95 | 2,695 | 6,785 |
| Rye | 12.0 | 1.89 | 5.83 | 11.0 | 401 | 172 | 395 | 87 | 3,732 | 10,868 |
| Sorghum | 11.0 | 1.62 | 6.25 | 10.1 | 204 | 141 | 306 | 123 | 3,945 | 9,756 |
| Wheat | 12.0 | 2.09 | 5.83 | 12.2 | 374 | 196 | 382 | 142 | 4,280 | 12,607 |
| *Roots and tubers* | | | | | | | | | | |
| Potato | 78.0 | 0.32 | 6.25 | 2.0 | 96 | 26 | 75 | 33 | 667 | 1,572 |
| Sweet potatoes | 70.0 | 0.21 | 6.25 | 1.3 | 45 | 22 | 50 | 22 | 414 | 994 |
| Taro (*Colocasia*) | 72.5 | 0.29 | 6.25 | 1.8 | 70 | 24 | 74 | 26 | 707 | 1,737 |
| Yam (*Dioscoria*) | 72.4 | 0.38 | 6.25 | 2.4 | 97 | 38 | 86 | 30 | 821 | 2,009 |
| Cassava meal (*Manihot*) | 13.1 | 0.26 | 6.25 | 1.6 | 67 | 22 | 43 | 19 | 404 | 1,184 |
| *Legumes (pulses)* | | | | | | | | | | |
| Beans (*Phaseolus*) | 11.0 | 3.54 | 6.25 | 22.1 | 1,593 | 234 | 878 | 223 | 8,457 | 20,043 |
| Beans, Broad (*Vicia*) | 11.0 | 3.74 | 6.25 | 23.4 | 1,513 | 172 | 786 | 202 | 8,244 | 20,951 |
| Chick-pea | 11.0 | 3.22 | 6.25 | 20.1 | 1,376 | 209 | 756 | 174 | 7,802 | 19,290 |
| Cowpeas (*Vigna*) | 11.0 | 3.74 | 6.25 | 23.4 | 1,599 | 273 | 842 | 254 | 8,640 | 21,086 |

TABLE 6. Imports of cassava starch and flour to Canada and the U.S.A. (Canadian imports include some sago flour (source: National Trade Data)).

| | U.S.A. | | Canada | |
|---|---|---|---|---|
| | Million lb | Million $ | Million lb | Million $ |
| 1964 | 294 | 9.6 | 7 | 0.5 |
| 1965 | 358 | 12.2 | 10 | 0.6 |
| 1966 | 341 | 11.5 | 13 | 0.7 |
| 1967 | 304 | 10.7 | 20 | 1.1 |
| 1968 | 194 | 7.1 | 16 | 0.9 |
| 1969 | 195 | 6.8 | 15 | 0.8 |
| 1970 | 207 | 7.0 | 20 | 1.0 |
| 1971 | 182 | 7.1 | 9 | 0.6 |

in cassava starch gives it great dimensional strength, which makes it in particular demand for sizing paper or fibers, to give them greater tensile strength.

Cassava starch also provides good parent material from which to hydrolize dextrins for formulating adhesives. Such adhesives made from cassava appear to have a greater flexibility and less brittleness at low humidities than dextrins derived from cereal starches. Cassava starches also possess specific characteristics that are in demand in the food industry.

At the present time the United States is the principal user of cassava starch and imports around 90,000 tons/year (Table 6).

## Feed Use

Cassava has been used as a livestock feed on subsistence farms for many years, although there has been a traditional prejudice against its use in some areas because of the toxicity attributed to its cyanogenic glucoside content. However, the literature on this subject has tended to be inconclusive

TABLE 7. Comparison in prices of barley, maize, and manioc in EEC in September 1967 (U.S. $/ton) (source: GATT 1968 The markets for manioc).

| | CIF price | Import levies | Threshold prices or prices after levies paid | Difference | |
|---|---|---|---|---|---|
| | | | | Barley | Maize |
| Barley | (59.65) | 30.65 | 89.00 | | |
| Maize | (57.25) | 31.03 | 88.28 | | |
| Manioc chips | 61.60 | 5.52 | 67.12 | −21.88 | −21.16 |
| Manioc pellets | 64.40 | 5.52 | 69.92 | −19.08 | −18.36 |
| Manioc meal | 56.00 | 8.02 | 64.02 | −24.98 | −23.26 |

and controversial. Recent work by Maner (1972) and his co-workers clearly demonstrates the potential of high cassava rations. Only in the last decade has cassava assumed any significance as a component of compounded animal feeds where it is used in place of feed grains. This situation has arisen mainly because cassava enters the European Common Market at a highly favourable tariff rate compared to wheat, maize, and other energy components of compounded animal feeds (Tables 7 and 8).

At present the main users of cassava are Germany, Holland, and Belgium. France has a low usage because her agriculture is still in the process of modernization, the animal feed industry is backward, and large surpluses of cereal grains exist. Italy at present still benefits from low-cost maize feeds (which theoretically are not permitted under the common agricultural policy of the European Economic Community), and the United Kingdom, Eire, and Denmark have developed their feed industries on low-cost cereals which can be bought at a lower price than cassava in the world market, but not within the framework of the EEC Common Agricultural Policy.

TABLE 8. Relationship of prices for manioc, maize, and barley on basis CIF Rotterdam resp. FOB incl. tax and levies (source: Phillips unpublished data).

| | Maize | Manioc pellets | Feed barley |
|---|---|---|---|
| Average prices: | | | |
| 1968 World Market Price | 100.0 | 100.3 | 103.7 |
| EEC price | 177.3 | 126.0 | 174.0 |
| 1969 World Market Price | 100.0 | 93.6 | 81.9 |
| EEC price | 172.3 | 112.1 | 163.8 |
| 1970 World Market Price | 100.0 | 101.1 | 84.6 |
| EEC price | 148.2 | 114.4 | 148.0 |
| Maize World Market Price = 100 | | | |

In the last decade the importation of cassava to the countries of the European Economic Community has more than tripled (Table 9). Between 80 and 90% of the world market is supplied by Indonesia and Thailand. The latter country is a very small consumer of cassava and produces mainly for the world market (Table 14). Brazil, China, Tanzania, Malawi, and Angola also supply the world market to a much smaller degree (Fig. 4). At the present time 80–90% of the world trade in cassava for feeding purposes is absorbed by the EEC.

The use of cassava in compound feeds in the developing countries does not appear to have received any attention. This may seem surprising since the 1.5 million tons of cassava reaching the

TABLE 9. Imports of dried cassava products into the European Economic Community 1962–70, in thousands of metric tons (source: International Trade Centre GATT).

| | 1962 | 1963 | 1964 | 1965 | 1966 | 1967 | 1968 | 1969 | 1970 |
|---|---|---|---|---|---|---|---|---|---|
| Germany | 366 | 387 | 462 | 520 | 702 | NA[a] | 481 | 548 | 591 |
| Netherlands | 1 | 5 | 17 | 76 | 96 | NA | 237 | 444 | 502 |
| Belgium | 23 | 72 | 105 | 100 | 70 | NA | 127 | 212 | 268 |
| France | 23 | 20 | 18 | 17 | 16 | NA | NA | NA | 35 |
| Italy | 0 | 0 | 0 | 1 | 0 | NA | NA | NA | 14 |
| Total: | 413 | 484 | 602 | 714 | 884 | | 845 | 1204 | 1410 |

[a]NA, data not available.

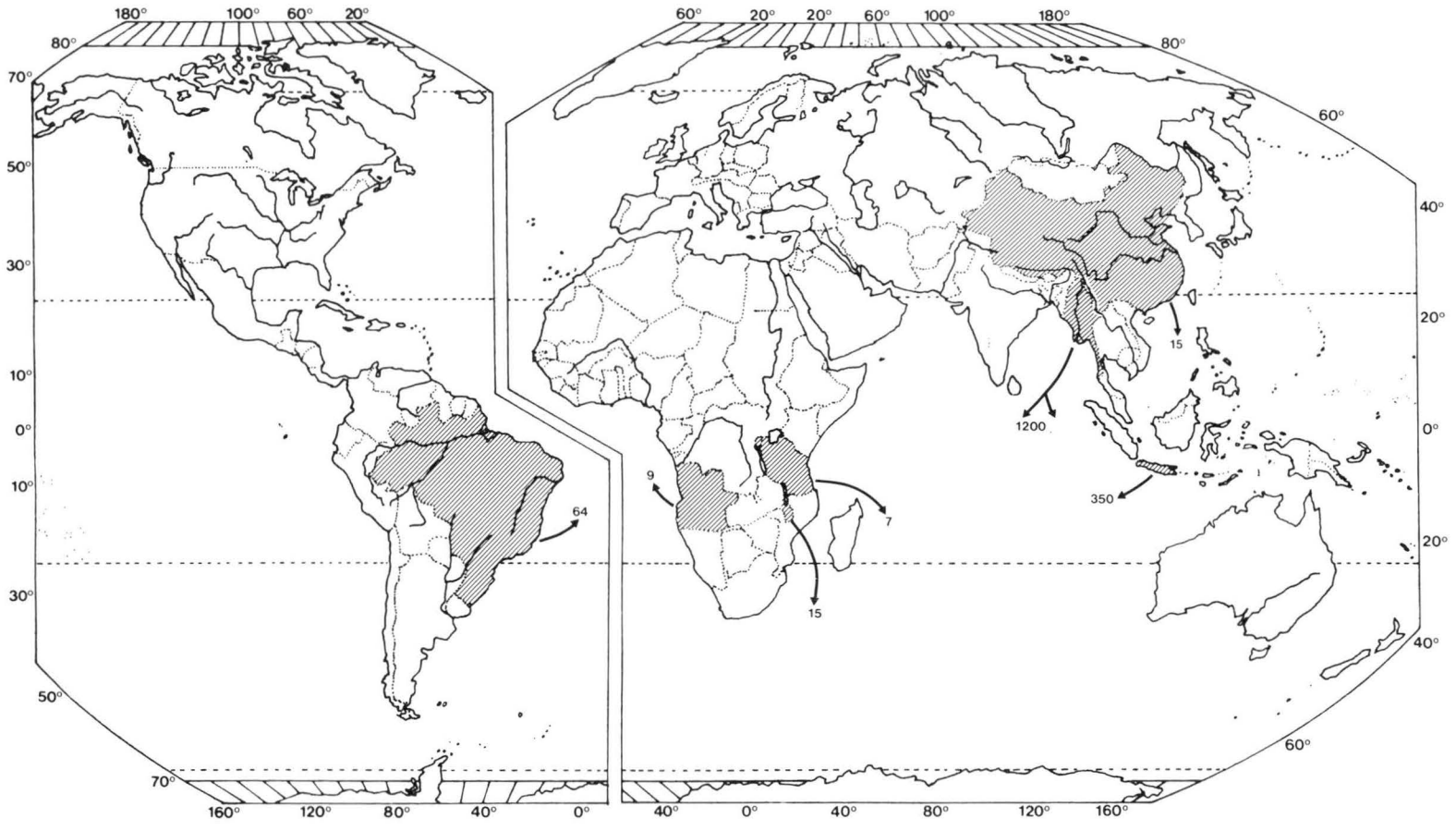


FIG. 4. World trade in cassava (in thousands of metric tons) in 1970.

European mills at about U.S. $75/ton has about 30% of its price made up of shipping charges from the Far East. In fact, the FOB cost at the dockside in Thailand and Indonesia appears to be less than U.S. $50/ton, a price considerably less than that at which cereals of similar energy value can be purchased for use in feed compounding in most developing countries.

While it is not possible to fully explain this situation, it would appear that the feed industry in developing countries is, to a large extent, owned and directed by multinational corporations who prefer to rely on known technology rather than to invest in the development of new technologies appropriate to the typical developing country situation (Johnson 1970). Within the EEC the size and fiercely competitive nature of the market is such that its skilled compounders appear to be prepared to utilize alternative energy sources in an innovative manner.

## Prospects for the Future

Cassava has a very low income-elasticity of demand. This means that when personal incomes grow people tend to spend very small parts of the increase on cassava. In fact, when their incomes increase considerably, they actually tend to shift their consumption from cassava to cereal grains. However, at the income levels encountered in cassava-eating areas, this change takes some time to occur (Fig. 5).

Because of this situation, the global food demand for cassava is likely to increase at a rate very similar to that of the human population of cassava-eating areas. In Table 10, two projections for the demand for cassava in 1980 are shown. The first represents a continuation of the past trend in demand and the second represents a projection based on a higher income growth than in the past, namely the one targeted for 1980 in the United Nations Second Development Decade studies. The difference between the two projections is very small for the reason that has already been noted. Both projections represent a growth rate in demand of about 2.6% per annum.

TABLE 10. Consumption demand for cassava in 1980 in thousands of tons (source: FAO 1972 unpublished data).

| | 1970 | 1980[a] | 1980[b] |
|---|---|---|---|
| World | 55,087 | 71,500 | 70,460 |
| Africa | 29,306 | 38,204 | 37,481 |
| Latin America | 8,492 | 10,838 | 10,651 |
| Asia and Far East | 16,422 | 21,318 | 21,154 |
| China | 734 | 971 | 1,007 |
| Rest of World | 133 | 169 | 167 |

[a]Demand projected on basis of past trend.

[b]Demand projected on basis of Second Development Decade growth model.

If we assume that there is some reduction in population growth in cassava-eating countries by the end of this century, it would appear that the demand at that time for cassava as a food will be not far short of 100 million tons. Even if we accept that cassava represents the food of low-income groups and that people will shift into cereals as and when their incomes permit, we are still likely to have twice as many food calories derived from cassava at the end of the century as are derived from cassava today. If we refer back to Table 3 we will see this means we can expect a very large number of cassava eaters in the year 2000. This suggests that the medical problems described in some of the papers presented in these Proceedings are not likely to be solved in the foreseeable future by a reduction in the use of cassava as a food.

The world starch industry is a fiercely competitive one in which cassava starch is only one of several available. The industry is dependent not only on production of starch but also on producing other products and by-products involving complex technology and marketing systems. Generally speaking, cassava processing in developing countries is carried out in a primitive fashion and the marketed product is often of poor and variable quality. In the absence of large infusions of capital the future prospects for cassava starch are questionable particularly insofar as it competes in many respects with starches being produced in the developed countries which represent the main outlet for the starch industry.

There are some prospects for the increased use of cassava flour as a partial substitute for wheat flour in composite breads in those countries where wheat flour is in limited supply. In recent years considerable progress has been made in overcoming the problems of finding: (a) appropriate additives to substitute for wheat gluten, (b) new mixing techniques to obtain improved gas retention and control of gelatinization during baking with non-wheat flours, and (c) appropriate meth-

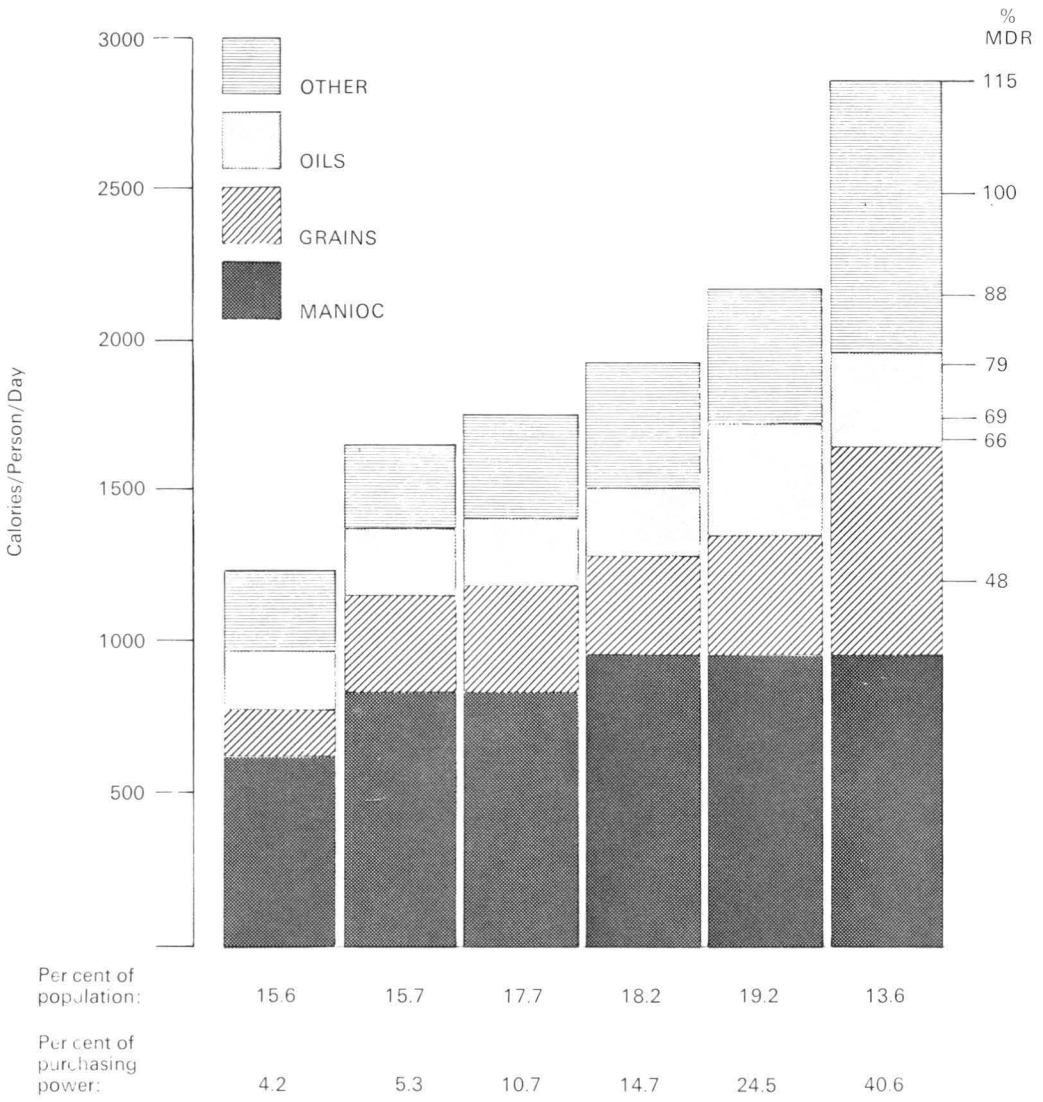


FIG. 5. Cassava consumption in relation to income levels in Zaïre (source: Rogers et al. 1971).

ods of protein fortification of such flours. Because of this progress the future prospects for expanded use of cassava flour in composite breads appear promising.

During the process of flour- and bread-making most of the cyanogenic glucosides appear to be destroyed and from the toxicity standpoint composite breads containing cassava flour may not be of any significance. However, since this use for cassava will likely become significant (bread containing up to 50% cassava flour has already undergone successful acceptability trials), some discussion of this point is warranted.

The really attractive area for future potential use of cassava seems to be in the compound feed sector. Table 11 shows the phenomenal way in which this sector has grown in recent years within the six original members of the EEC. A similar pattern of growth in feed use is taking place in certain developing countries where incomes have reached the stage where people can afford intensively produced meat. For example, Taiwan's

TABLE 11. Compound feed production in the EEC from 1955 to 1970 and percentage of increase in thousands of tons (source: EEC).

| | Belgium and Luxembourg | France | Germany | Italy | Netherlands | Total EEC |
|---|---|---|---|---|---|---|
| 1955 | 993 | 1,270 | 1,968 | 380 | 2,900 | 7,511 |
| 1960 | 1,550 | 2,220 | 3,578 | 800 | 4,600 | 12,746 |
| 1965 | 2,527 | 4,544 | 6,594 | 2,600 | 5,625 | 21,290 |
| 1967 | 3,119 | 5,847 | 7,723 | 2,500 | 6,392 | 25,316 |
| 1968 | 3,240 | 5,516 | 7,872 | 3,100 | 6,838 | 26,566 |
| 1969 | 3,668 | 6,244 | 8,863 | 3,300 | 7,117 | 29,192 |
| 1970 | 4,282 | 6,475 | 9,727 | 3,633 | 7,851 | 31,968 |
| % increase 1955–70 | 331 | 410 | 394 | 856 | 171 | 326 |
| Yearly increase % avg 1961–65 | 10.3 | 15.5 | 13.0 | 20.3 | 4.1 | 10.8 |
| % avg 1965–70 | 9.0 | 4.9 | 8.3 | 12.9 | 6.8 | 8.6 |

importation of feed grains has increased from 94,000 tons in 1964 to more than 1 million tons in 1971 and, as yet, shows no sign of levelling off.

The projected demand for cereal grains, and their substitutes as energy sources for livestock feeds, is expected to grow globally at a rate approaching 3%/year (Table 12). Although a substantial part of this growth will take place within the developing countries, an even greater part is expected in the developed countries and in the centrally planned economies, and this would appear to represent a particularly promising opportunity for export market development in a number of tropical countries.

At present the export of cassava and its products produces about $80 million of foreign exchange for the developing countries. However, most of this money flows to only two countries, Thailand and Indonesia. It is earned by exporting around 1.5 million tons of dried cassava products which represents about 5% of total world fresh cassava production. These figures are relatively small in terms of the export levels of some other tropical commodities (Table 13) although it would appear that a 6- to 10-fold increase in cassava exports by 1985 might easily be absorbed in the world feedstuffs market. At present price levels such an increase in exports would put cassava next to coffee and sugar as the most important agricultural export from the developing countries.

While such an increase in exportation may seem fanciful at first glance, recent computer studies of the compound feed market undertaken in Germany, England, and Canada (A. Hone, Institute of Commonwealth Studies, Oxford, personal communication) indicate that at its present price level,

TABLE 12. Estimated demand for grains for feed use, in millions of tons (source: FAO 1969 Indicative World Plan).

| | 1962 | 1985 |
|---|---|---|
| Developed countries | 202 | 320 |
| 64 developing countries | 17 | 48–68 |
| Centrally planned countries (excl. China) | 52 | 126 |
| Total: | 271 | 494–514 |

TABLE 13. Exports from developing countries of selected agricultural products, 1965–67 (source: FAO 1969 Indicative World Plan).

| | Millions U.S. $ |
|---|---|
| Sugar | 1109 |
| Wheat and coarse grains | 771 |
| Beef and veal | 321 |
| Citrus fruits | 194 |
| Coffee | 2167 |
| Tea and mate | 547 |
| Cocoa | 477 |
| Bananas | 411 |
| Cassava chips, pellets, and starch | 80 (1971 approx) |

TABLE 14. Utilisation of cassava in selected countries, 1964–66 (source: FAO Food Balance Sheets 1964–66).

| | Total production (*million tons*) | % consumed | % used as animal feed | % exported | % as "waste" |
|---|---|---|---|---|---|
| India | 3.1 | 93 | 0 | 0 | 7 |
| Zaïre | 7.2 | 95 | 0 | 0 | 5 |
| Nigeria | 7.5 | 80 | 0 | 0 | 20 |
| Indonesia | 11.1 | 88 | 2 | 9 | 10 |
| Brazil | 24.7 | 35[a] | 39 | 1 | 20 |
| Thailand | 1.6 | 39 | 0 | 56 | 5 |

[a]35 as food, 5 for non-food industrial use.

and assuming that the EEC Common Agricultural Policy does not discriminate specifically against cassava, a market demand of 4.3 million tons (almost triple the 1970 level) may be expected in the EEC by 1980. These studies also indicate that there may be some difficulty in supplying this market unless either the West African producers, who have preferential access to the EEC, or mainland China (whose production potential is not known), become significant exporters.

These projections do take into account expected increases in the demand for cassava in Italy and France (due to developments in their livestock industry) and in the three new EEC members (which will have to give up cheap imports of feed grains by 1977 to conform to the Common Agricultural Policy), but they disregard the Japanese market. Until recently Japan appears to have relied heavily on imported maize as its main source of feed energy, and indeed the Japanese feed market has played an important role in the development of the Thai and Philippine corn industries. Japanese buyers now appear to be active in the cassava market (T. P. Phillips, University of Guelph, personal communication) especially in Brazil where the growth potential is enormous. If Thailand can build up a 1.2 million ton dried cassava export in a decade it is not unreasonable to expect that Brazil (whose current production of cassava is reported to be at least eight times that of Thailand) could also become a major exporter. Other countries, such as Malaysia, are also already active in attempting to enter this export market.

I have particularly stressed the export market potential because beef, and to a lesser extent feeds for livestock, represent the only commodities for which a really strong export growth potential appears to exist for the products of developing countries. However, if we assume that at least part of the projected demand for cassava in the developed markets is realized, it would not be unreasonable to assume a partial spin-off in the development of the use of cassava for compound feed in the developing countries themselves within the next decade. Such a development which obviates shipping costs should be of particular interest to countries which currently import animal feeds.

If we bear in mind also that cassava is a commodity to which very limited research has been applied in the past (two major international agricultural research centres, in Colombia (CIAT) and Nigeria (IITA), are now giving priority attention to this crop), it is not unreasonable to expect that within the next few years we may see some pay-off for this research in the form of the development of production systems which will result in increased yields at lower unit costs. This in itself would be likely to increase the utilization of cassava as animal feed both in developed and developing countries.

Bearing in mind both the current role and growth potential that appear to exist for the use of cassava, a better understanding of the toxic role of its cyanogenic glucosides is both desirable and necessary. We hope that this Workshop will contribute towards such an understanding.

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# Cassava as Food: Toxicity and Technology

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COURSEY, D. G. 1973. Cassava as food: toxicity and technology, p. 27–36. *In* Chronic cassava toxicity: proceedings of an interdisciplinary workshop, London, England, 29–30 January 1973. Int. Develop. Res. Centre Monogr. IDRC-010e.

**Abstract** The toxicity of cassava (*Manihot* spp.) is caused by the presence of the cyanogenic glycoside linamarin, together with much smaller amounts of the closely related lotaustralin. These substances hydrolyse under the influence of the endogenous enzyme linamarase to liberate hydrogen cyanide (HCN).

The quantities of toxic principle vary greatly between cultivars and, although the so-called "sweet" cultivars are generally of lower toxicity than the "bitter" ones, the correlation is not exact. Cyanide levels cannot be related to formal botanical taxa. Some variation in cyanogen content with ecological conditions of plant growth also occurs.

A wide variety of traditional food preparation techniques are used for processing cassava in different parts of the world and an important element in all of these is an attempt to reduce the cyanide content by liberation of the HCN either by solution in water or by volatilisation. These processes involve maceration, soaking, boiling, roasting, or fermentation of the cassava roots, or a combination of these processes. The amount of analytical data available on the efficacy of these processes is limited and generally unreliable. It appears that normally the greater part of the cyanide is liberated in such processes, but there are often substantial residual quantities which may well be sufficient to produce chronic toxic symptoms, and occasionally even acute poisoning, in those who consume large quantities of cassava products.

Little reliable information is available as to whether linamarin itself constitutes a toxic factor, or whether toxicity only arises from hydrolysis of this compound to free cyanide.

**Résumé** Le manioc doit sa toxicité à un glycoside cyanogène, la linamarine, et à des quantités beaucoup plus faibles de son proche parent, la lotaustraline. Ces substances s'hydrolisent sous l'influence de l'enzyme endogène linamarase pour libérer de l'acide cyanhydrique.

Les quantités d'élément toxique varient considérablement d'une culture à l'autre et, bien que les soi-disant cultures "douces" soient généralement moins toxiques que les cultures "amères", il n'y a pas d'étroite corrélation. Le niveau de cyanure ne peut pas, non plus, être associé à des taxa botaniques formels. Il existe une certaine relation entre le contenu en cyanogène et les conditions écologiques dans lesquelles la plante croît.

Le manioc est traditionnellement préparé sous une grande variété de formes dans différentes parties du monde. Un élément important de ces préparations vise à réduire le contenu en cyanure par libération d'acide cyanhydrique, soit par dissolution dans l'eau soit par volatilisation. Ces procédés incluent macération, trempage, ébullition, rôtissage ou fermentation des racines du manioc, appliqués seuls ou en combinaisons. Les données analytiques sur l'efficacité de ces traitements sont peu nombreuses et généralement peu fiables. Les procédés semblent en général libérer

la plus grande partie du cyanure, mais il y a souvent des quantités résiduaires substantielles, suffisantes pour causer une toxicité chronique et, occasionnellement, un empoisonnement aigu chez ceux qui consomment le produit en grandes quantités.

Le peu de renseignements fiables que nous ayons ne nous permet pas de déterminer si la linamarine est elle-même un élément toxique ou si la toxicité provient de l'hydrolyse de ce composé en cyanure libre.

## Cassava Toxicity

### Toxic Principle of Cassava

THE fact that cassava (*Manihot esculenta* Crantz) can be toxic must have been known to the Amerindians since the earliest days of its domestication, if not before. The first reference to cassava toxicity in western literature appears to be in the writings of Clusius (1605) and there are many subsequent references in the accounts of travellers in tropical America and Africa in the following centuries. The association of the toxicity with the presence of hydrocyanic acid (HCN) was first made by Henry and Boutron-Charland (1836), while the identification of the occurrence of the HCN in the form of a cyanogenic glycoside, originally named manihotoxin, is due to Peckolt (1886, quoted by Cerighelli 1955). This compound, the principal cyanogen of cassava, was subsequently (Dunstan et al. 1906) shown to be identical with the better-known glycosides phaseolunatin and linamarin of *Phaseolus* and *Linum* respectively. The presence of HCN in "sweet" and "bitter" cassava was established by Francis (1878) and investigated further by Carmody (1900), Collens (1915), and Turnock (1937) who concluded that HCN was the only poisonous substance in cassava. Linamarin is structurally 2-(β-D-glucopyranosyloxy)isobutyronitrile; under enzymatic or acidic hydrolysis it liberates free HCN, together with acetone and glucose. Earlier work on the occurrence of linamarin in cassava has been reviewed by Jones (1959), Wood (1965a), Johnson and Raymond (1965), and Oke (1968).

More recent studies (Butler 1965; Nartey 1968; Bissett et al. 1969; de Bruijn 1971) have shown that a small proportion, between 2 and 8%, of the total cyanogenic glycoside present in cassava tubers consists of a methyllinamarin, believed to be identical with lotaustralin. This substance hydrolyses under similar conditions to linamarin, to yield HCN, methyl ethyl ketone, and glucose.

The toxicity of cassava thus appears to arise from the presence of cyanogenic glycosides, which may hydrolyse readily to free HCN, and it is with this aspect that this paper is concerned. It should be noted in passing, however, that earlier work (Clark 1936; Turnock 1937; Johnson and Raymond 1965) also refers to the presence of a toxalbumin. Compounds of this class are typical of the Euphorbiaceae, to which cassava belongs, while Clark (1936) specifically mentions post-mortem indications suggestive of toxalbumins in studies of cassava poisoning. This aspect appears to have been neglected recently, and would repay further study.

### Occurrence of Cyanogenic Compounds

The cyanogenic glycosides are distributed throughout the cassava plant, but the concentration varies greatly between varieties, and also with climatic, edaphic, and cultural conditions. Numerous publications discuss the range of cyanogen content of the edible tubers; among the more useful are: Greenstreet and Lambourne 1933; Dean 1937; Raymond et al. 1941; Joachim and Panditesekere 1944; Bolhuis 1954; Oyenuga and Amazigo 1957; Wood 1965a; Johnson and Raymond 1965; Oke 1968, 1969; Sinha and Nair 1968; de Bruijn 1971. The normal range of cyanogen content is from 15 to 400 ppm, calculated as mg HCN/kg fresh weight but occasional samples as low as 10 mg/kg or over 2000 mg/kg (Rogers 1963) have been reported. Most commonly, cyanogen content falls between 30 and 150 mg/kg.

Cassava is often described as "bitter" or "sweet" according to the amount of cyanide present, but these are at best only approximate terms, and to attempt to associate cyanide levels with particular botanical taxa is quite incorrect (Bolhuis 1954). No exact correlation between sweetness or bitterness of taste can be made (Pereira and Pinto 1962). In general, bitter cassava has a high cyanide content while sweet cassava tends to have lower values, but there is a great deal of overlapping between classes, as is clearly illustrated by a graphical presentation of the results of Sinha and Nair

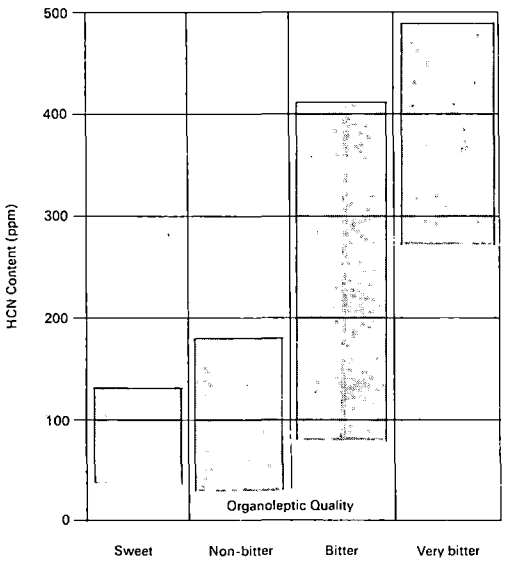


FIG. 1. Hydrocyanic acid content of different organoleptic categories of cassava (after Sinha and Nair 1968).

(1968) in Fig. 1. A substantial mythology has built up around the subject of cassava toxicity and bitterness, much of which has been demolished by Bolhuis (1954). In most varieties, under most cultural conditions, the concentration of cyanogenic glycoside is very substantially higher in the "peel" fraction of the tuber than in the flesh, the ratio being usually 5 or 10 to 1. As a rough guide to acute toxicity (Koch 1933; Bolhuis 1954; de Bruijn 1971), the following may be adopted:

*Innocuous*: Less than 50 mg HCN/kg fresh peeled tuber
*Moderately poisonous*: 50–100 mg HCN/kg fresh peeled tuber
*Dangerously poisonous*: Over 100 mg HCN/kg fresh peeled tuber

The factors responsible for sweetness or bitterness, apart from cyanogenic glycoside content, need further investigation. The free sugars normally present in cassava are glucose, fructose, sucrose, and traces of mannose (Ketiku and Oyenuga 1970) but the quantities would not normally be sufficient to cause a great degree of sweetness, although an early paper (Ewell and Wiley 1893) mentions sucrose content as high as 17%. The mannitol which occurs in cassava tubers may also play a part. However, as has been noted by Johnson and Raymond (1965), many of the publications dealing with the chemical composition of cassava are old and "in some cases the information cannot be substantiated." A thorough reexamination of the chemistry of the minor constituents of cassava could probably prove fruitful.

The cyanogenic glycosides of cassava are accompanied in the plant tissue by a hydrolytic enzyme, linamarase (occasionally known as linase), similar but not identical to emulsin (Armstrong and Horton 1910; Wood 1965b, 1966). In active, healthy tissue of the growing plant, enzyme and substrate do not come into contact, but contact occurs when the tissues are mechanically damaged, or when loss of physiological integrity occurs as a result of post-harvest deterioration of the tubers, or of wilting of the leaves; hydrolysis then takes place, liberating HCN.

## Nature of the Toxicity

Toxicity in cassava and its products is associated primarily with the free HCN in the material that is formed when the cyanogenic glycosides have been hydrolysed. To what extent the linamarin and methyllinamarin are themselves toxic to mammalian life is by no means clear. On the basis of a single rabbit experiment, Charavanapavan (1944) states categorically that linamarin is toxic, while Nijholt (1932) also suggests that hydrolysis of the linamarin can take place within the alimentary system, leading to poisoning. It has even been suggested (Boorsma 1905) that drinking water after a meal of cassava can increase the danger of poisoning. Other authors, such as Greenstreet and Lambourne (1933) and Montgomery (1969), consider that the toxicity of the linamarin, ingested as such, in the absence of linamarase, is unproven. General experience of the use of cassava-based foods would suggest that residual amounts of linamarin are at least not highly toxic, if the enzyme system has been deactivated, but this is a field which clearly needs further investigation. Although the toxicity of the glycosides present in cassava may be in doubt, HCN itself is one of the most powerful poisons known: large doses can cause acute poisoning, usually resulting in death, while the habitual ingestion of smaller quantities, even so small as to produce no immediate symptoms, can result in chronic effects. The division of cassava into "bitter" and "sweet" types relates to the likelihood of acute symptoms occurring after consumption of the product with-

out special care being taken to detoxify it. The classification already given is based on the statement of Boorsma (1905) that 50–60 mg HCN is a lethal dose for an adult male weighing 50 kg (Bolhuis 1954).

Acute poisoning as a result of eating cassava by man or domestic animals is not particularly common, but is by no means unknown; a considerable number of reports occur throughout the literature which will not be reviewed here. Chronic effects resulting from the continued ingestion of cassava products are probably a potentially much more serious factor limiting the use of cassava as human or animal nutrition. This aspect has been reviewed recently by Osuntokun (1972), and also forms the subject of other papers presented at this Workshop.

## Toxicity and Utilization

### Processing Requirements

Quite apart from any considerations relating to toxicity, cassava is normally processed in some way before being used. Unlike many other starchy staple foods, fresh cassava deteriorates extremely rapidly after harvest (Ingram and Humphries 1972), and any processing must therefore be undertaken within hours or at the most a day or two of harvest, unless the material is to be consumed immediately in the fresh state. A typical proximate analysis of peeled root (Winton and Winton 1935) is given in Table 1. The processes of post-harvest deterioration themselves result in hydrolysis of the glycosides present in the tuber, and as a result, stale cassava can be more toxic than fresh (Charavanapavan 1944; Greenstreet and Lambourne 1933).

In common with other starchy materials, processing usually by some form of heat treatment is necessary to render the material soft enough to be palatable. This involves heating the material to a temperature high enough for the starch to undergo gelatinization, either by boiling in water, or in the case of a high water-content material such as cassava, roasting or some similar treatment. In some cases, the heating is carried out at a higher temperature, causing a partial breakdown of the starch to dextrins. Occasionally, heat treatment is replaced by some form of soaking or steeping of the material in water, usually after shredding or other comminution. Under these conditions, a softening occurs in association with autolytic enzymatic processes.

TABLE 1. Proximate analysis of cassava (after Winton and Winton 1935).

| Composition of peeled root | % |
|---|---|
| Water | 61.3 |
| Protein | 0.6 |
| Fat | 0.2 |
| N-free extract | 36.5 |
| Starch | 31.0 |
| Fibre | 0.9 |
| Ash | 0.5 |

A second requirement for processing cassava is the need to eliminate, or at least reduce to acceptable levels, the toxic HCN, and the traditional processing techniques appear to be designed to do this.

### Traditional Processing Technology

A wide variety of techniques have been devised in various parts of the world to detoxicate the more poisonous varieties of cassava. Variants of most of these are to be found among Amerindian ethnic groups, and to some extent the spread of cassava utilization in other parts of the world has depended on the spread of these technologies—for example the transfer of the Amerindian technique for the production of "farinha de mandioca," via Brazilian negroes repatriated to West Africa, into the African technique for making the very similar "gari." In other cases, however, there has probably been independent invention on the processing technique after the crop has been introduced. For example, some of the African techniques involving shredding and soaking may derive from indigenous techniques for processing toxic yams (Jones 1959).

If linamarin is itself non toxic, presumably all that is necessary would be heating sufficient to denature the hydrolytic enzyme, and so prevent the release of free HCN. In practice, however, most traditional food preparation techniques appear to be designed to bring together enzyme and substrate by cell rupture, followed by elimination of the liberated HCN by either volatilization or solution in water. In some processes, the initial hydrolysis of the linamarin is assisted by the use of fermentation processes.

TABLE 2. A tentative classification of traditional cassava processing.

1. *No special detoxication techniques applied*
   - 1.1 Totally unprocessed (i.e. eaten raw)
   - 1.2 Simple cooking techniques only (as used for nontoxic starchy staples)
     - 1.21 Boiling, stewing, etc.
     - 1.22 Roasting, baking
     - 1.23 Frying
   - 1.3 Sundrying
     - 1.31 Sundrying without subsequent processing
     - 1.32 Sundrying with subsequent processing
       - 1.321, etc. Different types of milling, grinding, etc.
   - 1.4 Kiln or Hot-air drying
     (Subdivide as for 1.3)

2. *Special detoxication techniques applied*
   - 2.1 Detoxication by solution
     - 2.11 Soaking of whole roots or large pieces
       - 2.111 Soaking in static water
       - 2.112 Soaking in running water
       - 2.113 Soaking in salt water
     - 2.12 Soaking after comminution
       (Subdivide as for 2.11)
     - 2.13 Boiling
       - 2.131 Simple boiling
       - 2.132 Repeated boiling, in changes of water
     - 2.14 Wet extraction processes for starch
       - 2.141 Starch extraction without subsequent gelatinization
       - 2.142 Starch extraction with subsequent gelatinization
   - 2.2 Detoxication by fermentation
     - 2.21 Spontaneous fermentation
       - 2.211 Fermentation followed only by washing
       - 2.212 Fermentation followed by washing and heat treatment
         - 2.2121 Roasting
         - 2.2122 Steaming
         - 2.2123 Drying in hot air
     - 2.22 Fermentation with use of inoculum from earlier preparations
       (Subdivide as 2.21)

So great is the range of food products made from cassava and the diversity of the processes, that I have not been able to effect a complete classification. It is, however, the primary detoxication stage that is of greatest interest, and an attempt has been made (Table 2) to indicate the different types of *initial* process in the form of a taxonomic key. It is emphasized that this classification is tentative and probably incomplete. It is put forward as a first attempt to render some kind of order out of a widely distributed and somewhat chaotic literature. Account should also be taken of the classification of Schwerin (1971).

It is also emphasized that this scheme only classifies the earlier stages of the processes. For example, several of the different categories could lead to the production of some form of cassava flour, which, in turn, could be incorporated into a variety of porridges, doughs or bread-type or biscuit-type products. Most of the detoxication processes described are followed by some process for the reduction of the water content to a level which will permit the safe storage of the product. This level should be around 12% (Ingram and Humphries 1972), although this is not always attained in practice. The first stage of the drying process to achieve a level of around 50% usually involves physical expression of the water. This process was traditionally, in many Amerindian communities, accomplished by means of a tipiti

(Dole 1956), but in other parts of the world by the application of weights to the wet material contained in sacks or baskets, by various types of manual squeezing processes, by the use of mechanical presses (Jones 1959; Sturtevant 1969), or, in the case of industrial operations, by centrifugation (Akinrele et al. 1962). After this initial dewatering, the water content is reduced further by heating, which may also assist in driving off the last traces of free HCN.

Processes of group 1 (Table 2) can only be applied to cassava containing small amounts of glycoside—essentially the "innocuous" varieties in the Koch classification; only the "sweetest" varieties would be eaten raw. Nevertheless, all these processing methods appear to contribute very considerably to the reduction of available HCN in the tubers, and boiling, in fact, warrants a place not only here but also in group 2, as an actual detoxication technique. In some cases, within group 2, the distinctions made may be more philosophical than real. For example, some processes of steeping or soaking, especially in static water, may involve some degree of fermentation by extraneous microflora, at the same time that autolytic hydrolysis of the linamarin and the subsequent extraction by solution of the liberated HCN is taking place.

Even in the case of the typical fermentation processes, it is by no means clear to what extent the microorganisms responsible for the fermentation actually influence the linamarin content directly, or whether they serve merely to increase the acidity of the medium, thus aiding the endogenous enzymatic process.

It has been shown (Collard and Levi 1959; Collard 1963; Akinrele 1964) that in the fermentation of mashed cassava under conditions simulating the detoxication stage of gari manufacture, the *p*H of the medium falls during the first 24 h of the process under the influence of lactic and formic acid produced by *Corynebacterium manihot*. The level of *p*H 5 attained is, however, the optimum range for the action of endogenous linamarase and it may be that the effect of fermentative microorganisms is mainly in the acidification of the substrate.

The details of the methods of production of cassava-based food products in various parts of the world have been given by numerous authors. Among the most useful are: Adriaens 1942; Pynaert 1951; Jones 1959; Normanha 1969, 1970; Normanha and Pereira 1963; Sturtevant 1969; Ekandem 1961; Favier et al. 1969; Oke 1968; and Schwerin 1971; other references may be found in these papers, and in Ingram and Humphries (1972). The products used in the animal feedstuff industry are described in Anonymous (1968) and Maner (1972).

Most of these accounts are almost entirely descriptive, however, and contain little discussion of the biochemical and food technological parameters involved in the processes, and most are oriented to particular areas of the world.

There is a serious need for a major study, on a global basis, of traditional cassava processing technology, coupled with investigations, using modern analytical techniques, of the efficacy of the various processes in removing both glycosides and free HCN.

### Traditional Detoxication Methods

Although there is no doubt that fatal cases of poisoning due to ingestion of cassava do occur in both man and animals, and that the evidence for a causative link between habitual large intakes of cassava and various types of chronic degenerative conditions is strong, the fact remains that both these types of intoxication are comparatively rare. Millions of the world's population habitually consume cassava as a staple, and millions of tons of dried cassava products are incorporated into animal feed in European countries, apparently without complaint. This suggests that traditionally used detoxication processes are, in general, very effective. A problem certainly exists, but it must be kept in perspective.

Some of the earlier workers stated that properly prepared cassava products are free from toxicity. For example Vuaflart (1908, quoted by Oke 1968) says that hydrocyanic acid is "rarely present" in manioc flour, although he mentions an example of flour that contained 41 mg HCN/kg; samples of gari from various parts of francophone West Africa were reported (Vignoli and Cristau, 1950) as being free of HCN, as were samples of manioc flour from Brazil (Bethlem 1950, quoted by Oke 1968). Normal cooking methods were reported by Collens (1915) to remove all HCN from samples of sweet cassava, but with bitter varieties the cooked product still contained 20 mg HCN/kg, and even sweet varieties showed the presence of up to 17 mg HCN/kg after having been left overnight in the

water used for boiling. Nemoto (1940) categorically states that when manioc flour containing a little HCN is used in breadmaking, "all trace of HCN is removed," while Paula and Rangel (1939) state that the best flours are free from cyanide. Little attention seems to be paid by the animal feeds industry to cyanide content in dried cassava products. Of the several published standards for such products, only the Indian Standards Institution (1959) mentions the subject of HCN level, and sets a limit as high as 300 mg HCN/kg.

There is extremely little published information on HCN levels in actual cassava food products, and little of what there is relates these levels to the initial level in the unprocessed root. Simple drying of the sliced or rasped root was shown by Charavanapavan (1944) to be capable of removing up to 90% of the HCN when the drying was conducted at 60°C, but drying at temperatures approaching 100°C was less efficient; this latter result is not surprising, as drying at such elevated temperatures could denature the enzyme systems, and prevent autolytic hydrolysis of the glycosides taking place. However, Paula and Rangel (1939) reported the opposite effect, material initially containing 39 mg HCN/kg being reduced to sun-dried product at 17 mg HCN/kg, but by oven-drying to 6 mg HCN/kg. Results quoted by Joachim and Panditteseker (1944) indicated a loss of only one third of the total HCN present on drying at the controlled temperature of 60°C and even lower losses at higher temperatures. The results obtained by Razafimahery (1953) indicate however that about two thirds of the HCN present was lost during sun-drying for 7 days. Kokonte (a flour prepared from sun-dried chips) contains 20 mg HCN/kg (Wood 1965b).

Simple boiling of the roots reduces the HCN levels very considerably. A variety originally containing 332 mg HCN/kg (Raymond et al. 1941) contained only 10 mg HCN/kg after boiling; Paula and Rangel (1939) detected no HCN in a variety originally containing 39 mg HCN/kg after only 10 min boiling; the observations of Collens (1915) have already been mentioned. The effect of boiling on a number of varieties ranging from 103 to 232 mg HCN/kg fresh was studied by Joachim and Panditteseker (1944); the boiled products ranged from 27 to 87 mg HCN/kg, with no particular correlation with the initial HCN content. The authors commented that the varieties which lost least toxicity were those that did not become soft and floury on boiling. They also showed that steeping in warm water for short periods before drying can greatly reduce the HCN levels, especially if the material is grated. The Madagascan food product Bononoka, prepared by steeping the roots in running water for several days, followed by steaming, is free of HCN (Razafimahery 1953).

The HCN levels (in milligrams HCN per kilogram) of samples of a number of African cassava-based food products have been given by Oke (1968): Fresh Cassava 380, Gari 19, Fufu 25, Lafun 10, and Kpokpogari 11. Wood (1965b) gives a value of 25 mg HCN/kg in fresh gari, falling to 2 mg HCN/kg after storage for 1 month. During the extensive investigation conducted in Nigeria in connection with the mechanised production of gari (Akinrele et al. 1962) a value of 30 mg HCN/kg was regarded as acceptable in the product. In Brazil, where cassava flours are extensively used in the baking industry, Paula and Rangel (1939) state that crude flours made from bitter cassavas contain 10–200 mg HCN/kg, but the better grades of table flour are free from HCN; Nemoto (1940) gives 27–37 mg HCN/kg as the normal range, but that grated manioc flour may contain as much as 125 mg HCN/kg. Both reports state that these residual quantities of HCN are destroyed in the baking process when the flours are incorporated in bread. However, in studies on the manufacture of rotis (a roasted product) with cassava flours containing 57–118 mg HCN/kg, Joachim and Panditteseker (1944) found losses ranging from 47 to 80% during cooking, which would indicate a fairly high degree of retention.

It thus appears that cassava food products, as commonly prepared by many of the various detoxication methods outlined in Table 2, can still contain appreciable traces of HCN. The cases of acute poisoning that are reported probably have arisen after the consumption of products which have been carelessly prepared, or from unusually high HCN material, by operators who are more familiar with less toxic varieties, and/or have been taken in meals components of which contained active enzymes capable of hydrolysing linamarin. There may also be variations in individual susceptibility to HCN poisoning, while an abnormal gut microflora might lead to any unusually high release of hitherto unhydrolysed linamarin, after ingestion. In the context of chronic cassava toxicity, however, the traces of cyanide that appear

frequently in cassava-based foods probably constitute a more serious problem.

Quite apart from the small amount of analytical data that exist on the HCN content of food products, doubt must be cast on the reliability of some of the analyses, especially the older ones—an observation that also applies to data on the HCN contents of fresh material. As pointed out by Joachim and Panditteseker (1944) "the standard method for the estimation of HCN in materials containing cyanogenetic glucosides gives low results with manioc and its products." They found that the amount of HCN released autolytically in the analysis increased very considerably with the period allowed for autolysis up to 24 h, while even when the autolysis was essentially complete, a further quantity of HCN could be released by acid hydrolysis. Similar observations were given by Normanha (1965).

More reliable methods have been developed for the assay of both glycoside and HCN (Wood 1965b, 1966) and there have been alternative approaches to the subject (Adriano and Ynalvez 1932) some of which have been reviewed by Oke (1969). However accurate the analysis for HCN in the extract from the material may be, the accuracy of final result will be prejudiced when only incomplete liberation of HCN from the experimental material takes place. Cassava tuber is such a variable material that it is necessary to pay exceptionally close attention to the sampling regime, if anything approaching a true sample is to be obtained.

The incomplete release of HCN that takes place in analytical procedures also indicates that, under traditional food technology processes similarly aimed at achieving autolytic liberation, it is likely that not all the glycosides present are broken down. This was indeed anticipated as early as 1900 by Carmody who found that several successive water extractions each removed further quantities of HCN from the tubers. It is not, therefore, in any way surprising that cassava food products, even when carefully prepared, contain some residual HCN, still in bound form, which can under certain circumstances be liberated. The amount and quality of the analytical data available in the published literature do not permit a very detailed appreciation of the situation, but such indication that can be given would be that at least many cassava-based foods make appreciable contributions of HCN to those diets in which they are consumed in large quantities and that an association with chronic symptoms of HCN poisoning, especially in highly susceptible individuals, cannot be ruled out.

## Suggestions for Further Investigations

Although the evidence reviewed is suggestive, it is too inadequate to be definitive, and a program of food technological investigation should be initiated in this field. The following points would appear worthy of attention:

1. Evaluation of existing analytical techniques for the determination of glycosides, glycosidases, and free HCN in cassava and its products, and the refinement of these techniques;
2. The study, initially from the literature, but also later from direct field observations, of traditional detoxication techniques, and their critical evaluation using reliable analytical procedures. The key to the classification of such techniques given here may be useful, but it may well be modified in the course of this investigation;
3. A definitive examination of the degree to which unhydrolysed linamarin and lotaustralin are toxic when ingested in the absence of active linamarase;
4. A study of reported cases of acute toxicity of cassava or its products in both man and domestic animals, with the aim of determining the factors involved;
5. A study of the hydrolysis of linamarin and lotaustralin by linamarase in model systems, in the hope of defining the reasons for the incomplete hydrolysis that occurs during at least some detoxication processes;
6. A re-examination of the chemistry of the minor constituents of cassava, with a view to detecting toxic substances, other than cyanogens.

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# Cyanide Toxicity in Relation to the Cassava Research Program of CIAT in Colombia

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**Abstract** Despite the fact that an estimated 8–10% of the global daily caloric needs of man are supplied by cassava, the crop has not been subjected to any concentrated research effort to advance the technology of its production. A serious shortage of calories in the tropics is noted and in response to this need, CIAT has undertaken a full-scale program to develop cassava as an efficient producer of calories for human consumption, as well as for the growing animal feed and industrial starch markets. CIAT research concentrates on increasing yields, lowering production costs, and developing simple storage and processing methods.

The importance of cyanide toxicity is noted as is CIAT's intention to screen its large germ plasm collection for a cultivar with a zero or very low cyanide level. However, CIAT does not intend to carry out research to develop methods of detoxification.

Relationships between cassava cyanide content and nitrogen fertilization are discussed as well as the affinity of certain insect pests to secondary chemicals associated with the cyanide.

**Résumé** En dépit du fait qu'environ 8–10% des calories requises chaque jour par le monde entier proviennent du manioc, cette culture n'a jamais été l'objet de recherches concertées en vue d'en améliorer les techniques de production. Réalisant les déficiences sérieuses de calories dans les régions tropicales, le CIAT a mis sur pied un programme complet visant à développer le manioc comme source efficace de calories, tant pour la consommation humaine que pour répondre aux exigences de marchés toujours croissants de moulées à bétail et d'amidon industriel. Le CIAT oriente ses recherches vers l'augmentation des rendements, l'abaissement des coûts de production et le développement de méthodes simples d'entreposage et de traitement.

L'auteur note l'importance de la toxicité du cyanure, et fait part de l'intention du CIAT de trier sa volumineuse collection de plasmas germinatifs en vue de sélectionner une variété à teneur nulle ou faible en cyanure. Cependant, le CIAT n'a pas l'intention de poursuivre des recherches dans le but de développer des méthodes de désintoxication.

Il examine les relations entre le contenu en cyanure du manioc et l'emploi de l'azote comme engrais, de même que l'affinité de certains insectes nuisibles pour les substances chimiques secondaires associées au cyanure.

THE number of people in the world dependent on cassava as a staple food is not accurately known. Estimates vary from 200 million (Coursey and Haynes 1970) to 300 million (FAO 1968). Hendershott et al. (1972) estimated that cassava alone contributes 8–10% of the daily global caloric needs of man. Cassava, unlike the other main carbohydrate producers, wheat, maize, rice, and potatoes, is grown almost exclusively (97%) in the tropics (Gutierrez and Andersen 1972).

Because of this, cassava has not received the concentrated attention from scientists in the developed countries and hence the technology associated with its production is not advanced. This does not imply that the scientists in the developing countries have not applied themselves to the task of advancing this technology; in fact, a great deal of practical and useful knowledge has been compiled, notably in Brazil. However, there has never been at any one time a sufficient concentration of effort and resources to gain the knowledge of the plant required to really advance the technology. An example is thrips (*Euthrips* spp.) infections which we found causes damage very similar to "witches broom," a mycoplasma disease; the symptoms of this infestation have apparently never been attributed to this insect in the past.

Nevertheless, in spite of this general lack of knowledge cassava has often been quoted as a potentially efficient producer of calories. De Vries et al. (1970) suggested, after reviewing productivity of various crops, that cassava is probably potentially the most efficient calorie producer of the major crop species. Recently, there has been a tendency to regard protein as the major nutrient need of tropical zones. Although protein is very important, it should also be noted that there is a serious shortage of calories. Gutierrez and Andersen (1972) estimate that only 68% of the required calories are at present available in the tropics. When viewed in the light of the alarming increases in population, it seems evident there is a great need for an efficient producer of calories. Cassava can also be used as an animal feed or as a producer of starch for industrial use.

## CIAT Program

The CIAT Board of Trustees approved a full-scale cassava program in CIAT in June 1971. This program has as its objectives: 1) to gain more knowledge on methods of production and utilization of cassava, and 2) to train people to adapt these methods to their own locality and to train people to spread this knowledge. CIAT recognizes that there are many problems associated with both production and utilization of cassava; the major ones as seen by us are: 1) Low yield; 2) High production costs; 3) Deterioration in storage; 4) Toxicity; 5) Low nutritional quality as a food; and 6) Lack of general information.

CIAT is concentrating its research efforts on increasing yields, lowering production costs, and simple on-farm methods of processing (e.g. drying), and storage. Although, of course, the importance of cyanide toxicity is well recognized it does not form a major part of the CIAT Cassava Program.

## Implications of Cyanide

The problem of cyanide toxicity as a factor limiting utilization of cassava is very important. At present most cassava produced is for direct human consumption and undoubtedly most will be consumed by this group for some time to come. Philipps (personal communication CIAT-Guelph economic studies) has tentatively suggested that in the future there may be greater consumption of cassava in the livestock industry, particularly if production costs can be lowered and a better-quality dried product produced. Therefore the toxicity problem cannot be ignored if cassava is to make up a large portion of animal feedstuff, and the future potential of cassava for industrial use is to be realized. However, in the latter case high cyanide levels do not seem to be of importance. In fact, Holleman and Aten (1956) suggested that high cyanide content may be advantageous since it prevents stealing of the fresh roots from the field.

There is sufficient evidence being compiled (Ekpechi et al. 1966; Osuntokun et al. 1969; Makene and Wilson 1972) to suggest that chronic cyanide poisoning is of great importance in areas where cassava constitutes a major portion of the diet. At present CIAT does not have sufficient expertise to study this subject and also that of methods of detoxification.

Nevertheless, we recognize the need for research on these problems. CIAT probably has the largest collection of cassava germplasm in the world. This collection will be screened tor types of low toxicity. Before this is undertaken it is necessary to know if the effects of cyanide toxicity are solely due to cyanide residues in the material to be eaten, or if they are also associated with hydrolysis of glycosides by acids or enzymes present in the human alimentary tract. Montgomery (1969), in a review of cyanogens, stated that the "ultimate fate of ingested cyanogenetic glucoside remains unknown, and it may quite possibly lead to an appreciable increase in the body's cyanide or

thiocyanate pool." Charavanapavan (1944) reported the death of rabbits after feeding an aqueous solution of the cassava glucoside free of enzymes.

Makene and Wilson (1972) have suggested that the levels of linamarin in varieties should be reduced. Hence, it seems necessary to screen varieties both for low cyanide and low linamarin content, with the possibility of finding either a zero-cyanide or zero-glucoside variety. For example, in white clover (*Trifolium repens*) different types have been found containing: a) lotaustralin and linamarase, b) linamarase only, c) lotaustralin only, and d) neither lotaustralin nor linamarase. The presence of both the enzyme and the glucoside is controlled by different dominant genes (Corkill 1942). Bolhuis (1972) at a Cassava Program Review Conference in CIAT reported a zero-cyanide variety which unfortunately was lost during World War II. It is not known whether this zero-cyanide variety was due to a lack of enzyme or glucoside or both. However, it encourages us to search for a zero-cyanide variety and even if this ultimate goal cannot be achieved there is very large variation in the cyanide content of different clones of cassava from which low-cyanide types can be selected (Monclova 1936; Bolhuis 1954; Pereira et al. 1960; Chadha 1961; Pereira and Pinto 1962; Wood 1965; Barrios and Bressani 1967; Bostero 1968; de Bruijn 1971).

The means of screening these varieties for low- rather than zero-cyanide content depends on adequate testing techniques. Cyanide content apparently does not vary much with the age of the plant, but varies with mineral nutrition (nitrogen increases and potassium decreases the cyanide levels), shading of the plant, and water stress (de Bruijn 1971). In spite of variations due to climatic conditions the CIAT collection can be screened crudely to find potentially low-cyanide types without regard to these factors. These low-cyanide types can then be studied in more detail.

It is unfortunate that increasing levels of nitrogen fertilizer increase cyanide levels (de Bruijn 1971) as improvement of cultural practices will almost certainly be associated with the use of more nitrogen. The use of this technology should not be relinquished so as to lower levels of cyanide.

Although Holleman and Aten (1956) suggest that bitter varieties are higher-yielding than sweet ones there appears to be no strong evidence to suggest that this is universally true. We believe that high-yielding sweet varieties can be obtained, since two of the most promising lines being tested at CIAT, M Col 22 and Llanera, have HCN levels of 60–80 ppm.

It has been suggested that the high cyanide level in cassava may be correlated to insect resistance (Hendershott et al. 1972). However, in a general review of plant resistance to insects, Beck (1965) suggests that secondary chemicals, such as the glucosides, may actually act as attractants or stimulants to the insect pests. In the case of the Mexican bean beetle (*Epilachna varvivestris*), lotaustralin and phaseolutin act as feed attractants and stimulants (Nayar and Fraenkl 1963). It is then possible that by breeding for very low or zero cyanogen levels the insect resistance of these plants might actually be enhanced. Hence, we do not feel that the requirements of low or zero cyanogen are conflicting with high levels of insect resistance.

One can only speculate on the effects that cyanogens may have on disease resistance. However, so far at CIAT there seems to be no obvious difference in resistance between clones with different cyanide content.

## Conclusions

In conclusion, CIAT is looking for low or zero cyanide or linamarin varieties of cassava to be used in its breeding program so as to eliminate the problem of chronic toxicity. We do not feel that changes in cultural practices can usefully be utilized to reduce cyanide levels. CIAT will not search for new or improved methods of detoxification or attempt to investigate the mechanisms of toxicity. Nevertheless, the program is very dependent on gaining further knowledge especially concerning whether linamarin and lotaustralin are themselves toxic or only in the presence of linamarase, in order to clarify its selection criteria.

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# Cyanide Toxicity and Cassava Research at the International Institute of Tropical Agriculture, Ibadan, Nigeria

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**Abstract** Because of IITA's interest in cassava utilization by humans and livestock, selection for acyanogenesis will be an important objective of the cassava-breeding program. Selection for acyanogenesis will be based on finding cassava lines that lack the glucoside, glucosidase, and/or the glucoside and glucosidase. While stressing the importance of acyanogenesis, other important objectives of the program will be the retention of features such as resistance to insects and diseases in acyanogenic cassava plants.

**Résumé** A cause de l'intérêt que porte l'IITA à l'utilisation du manioc par l'homme et le bétail, un objectif important de son programme de recherche sur la génétique du manioc visera à la sélection de plants pour leur acyogénèse. A cette fin, on tentera de découvrir des lignées de manioc dépourvues de glucoside, de glucosidase ou des deux à la fois. Tout en plaçant l'emphase sur l'importance de l'acyogénèse, d'autres aspects importants du programme porteront sur la rétention, par les plants de manioc acyanogènes, de caractères tels la résistance aux insectes et aux maladies.

IN Africa, about 30 million tons of cassava are produced annually on about 5 million ha (F.A.O. 1971). This comprises about 35% of the world production and approximately 50% of the area devoted to this crop throughout the world. Approximately 80% of the production and 70% of the area of cassava in Africa is grown in tropical West Africa. In this region, cassava is the chief staple food and, therefore, is regarded as the most important among root and tuber crops.

Current yield of cassava is about 6 tons/ha. Under experimental conditions, yields over 65 tons/ha have been reported by Jones (1959). With the development of improved cultivars and agronomic practices, cassava yields are expected to be as high as 80 tons/ha. This is more than a tenfold increase. Most of the cassava is now grown as a subsistence crop in a shifting cultivation type of agriculture. The suitability of cassava to this type of agriculture is due to its drought tolerance and resistance to insects, rodents, and some diseases. But above all, and in contrast to other root and tuber crops, cassava roots store well in the soil where they were grown. This storage capability will probably diminish in the future as shifting cultivation of land gives way to continuous and more permanent cultivation. This means that in the next few decades, assuming our hopes for achieving higher yields are realized, outlets for the increased supply and new usages have to be developed.

Finding new outlets and usages for cassava will be difficult indeed if the danger of cassava cyanogenesis continues to loom in the horizon.

The task of marketing cassava products in international markets will be very difficult if the toxicity question is not fully understood.

This points out the urgent need for elaborate and immediate research on cassava cyanogenesis and finding methods for its elimination from the plant or its food products. To carry out such investigations, there is urgent need for reliable qualitative and quantitative methods for determining the glucosides, glucosidases and the liberated hydrocyanic acid (HCN). The methods have to be simple and not require complicated and expensive instrumentation. Such methods have to be fast to be suitable for screening large germplasm pool and breeding materials of cassava. To illustrate the problem, it should be mentioned that, at IITA, in 1972, we had 12,000 accession numbers of cassava. We hoped to test all of these lines for cyanogenesis. Unfortunately, that was not possible in the absence of a quick and reliable method to determine cyanogenesis. In 1973, we expect about 100,000 accessions from introductions from all around the world and their recombinations, which we hope to evaluate for cyanogenesis. Again it is very unlikely that such a task can be accomplished without the availability of new screening methods. We are currently attempting to develop a qualitative analytical method which is suited for large-scale screening.

Selection for acyanogenesis can be based on finding a cassava line that lacks one of the following: a) Glucoside, b) Glucosidase, or c) Glucoside and glucosidase.

The toxicology of the glucoside is unknown and opinions are conflicting. Jones (1959) indicated that the glucoside, if ingested without the enzyme from cassava, can still be hydrolyzed by enzymes that may be present in the intestinal tract or that may be introduced to it by eating uncooked fresh vegetables. Nicholls (1951) indicated that some people who eat large quantities of cassava refuse to eat uncooked food with it, suggesting that the proper enzyme may be introduced to the body from ingested raw fruits and vegetables. Due to the stability of the glucoside (Wood 1966) and its water solubility, its hydrolysis in the digestive tract by body enzymes or by exogenous enzymes from raw fruits and vegetables is very likely. Due to such uncertainties, our goal at IITA is to find lines that are completely acyanogenic because of the glucoside and the glucosidase.

In our breeding program, selecting for acyanogenesis is only one objective. We will stress acyanogenesis without jeopardizing other important objectives of the program. One important aspect that we have to keep in mind in selecting for acyanogenesis is the possible vulnerability of acyanogenic plants to insects, diseases, and field rodents. In the cassava root, a major portion of the glycoside is concentrated in the peel. This feature may help in protecting the root from field rodents and soil insects. On the other hand, the resistance of cyanogenic cassava types to insects such as locust may be related to the cyanogenic content of leaves. These suggestions are not well documented in the literature but, if true, their significance in providing protection to the plant cannot be underestimated. One of the major diseases of cassava is the cassava mosaic virus which is transmitted by the whitefly *Bemisia* spp. It is important to know the feeding preference of *Bemisia* to low or highly cyanogenic cassava, since if cassava plants are vulnerable to *Bemisia* because of low cyanogenesis, we would be defeating a major objective of our program. More work should be done concerning insect and disease susceptibility in regard to cyanogenesis as cyanogenesis could be the natural defensive mechanism making cassava so successful in tropical areas of the world.

Although our immediate interest is related to production aspects, future attention will be given to processing aspects. This will involve evaluating the efficacy and the improvement of present traditional methods of food preparation to free cassava from cyanogenic compounds.

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Wood, T. 1966. The isolation, properties and enzymic breakdown of linamarin from cassava. J. Sci. Fd. Agric. 17: 85–90.

# The Cyanogenic Character of Cassava (*Manihot esculenta*)

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**Abstract** Results of a study on cassava toxicity carried out in the Ivory Coast are presented. The distribution of the cyanogenic glucosides in the plant has been studied. It is concluded that classifying clones for toxicity according to the glucoside content of the tuberous roots is not strictly correct for other parts of the plant. Glucoside concentration of the leaves and of bark of tuberous roots of less toxic clones tends to be, on average, only slightly lower than in the same organs of very toxic clones.

Environmental conditions have a very important influence on the cyanogenic glucoside content of the tuberous roots. Different clones do not react in the same way to changing ecological conditions. Nitrogen fertilization increases, and supply of potassium and farmyard manure decreases the glucoside content. The influence of phosphate, calcium, and magnesium does not seem to be important. Drought increases glucoside content. Shading young plants increased the glucoside content of the leaves, but decreased that in the roots. No relation was found between the glucoside content of tuberous roots and the age of the plant. Glucoside concentration of a clone appears to be positively correlated with water content of leaves and tuberous roots, and a slight positive correlation with productivity was found.

There may be transportation of the glucoside in the plant. Ringing of stems caused a considerable increase of the glucoside content in the bark above the incision, and such an accumulation was not found when the leaves were eliminated before.

Distribution in the plant of the enzyme linamarase was studied. Activity is highest in the very young expanding leaves. In the bark of tuberous roots the activity is relatively very high, but in the inner part of the roots activity is very low. A knowledge of the distribution of linamarase activity offers possibilities for developing more effective methods for elimination of the toxicity of cassava products. The process of breaking down the glucosides of the grated inner part of the tuberous roots can be accelerated considerably by the addition of leaves or bark of tuberous roots, after which the hydrogen cyanide can be driven off.

**Résumé** L'auteur fait connaître les résultats d'une étude sur la toxicité du manioc, effectuée sur la Côte d'Ivoire. Il a étudié la distribution des glucosides cyanogènes dans la plante. Il en conclut que les clones taxonomiques de toxicité basés sur la teneur en glucosides des racines tubéreuses ne sont pas strictement applicables aux autres parties de la plante. La teneur en glucosides des feuilles et de l'écorce des racines tubéreuses de clones moins toxiques a tendance, en général, à être seulement un peu plus faible que celle des mêmes organes de clones très toxiques.

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Les conditions du milieu ont une très grande influence sur le contenu en glucosides cyanogènes des racines tubéreuses. Les clones ne réagissent pas tous de la même façon à des conditions écologiques changeantes. La fertilisation à l'azote cause une augmentation, alors que le potassium et le fumier de ferme produisent une diminution de la teneur en glucosides. La protection des jeunes plants contre la la lumière résulte en une augmentation du contenu en glucosides des feuilles, mais en une diminution de celui des racines. On n'a pas trouvé de relation entre le contenu en glucosides des racines tubéreuses et l'âge de la plante. Il semble y avoir corrélation positive entre la teneur en glucosides d'un clone et la teneur en eau des feuilles et des racines tubéreuses, et, à un degré moindre, la productivité du clone.

Il peut y avoir transport des glucosides dans la plante. Une incision annulaire des tiges produit une augmentation considérable du contenu en glucosides de l'écorce au-dessus de l'incision. Il n'y a pas accumulation lorsque les feuilles ont été enlevées au prélable.

La répartition de l'enzyme linamarase dans la plante a été étudiée. L'activité est maximale dans les feuilles très jeunes en voie d'expansion. Dans l'écorce des racines tubéreuses, l'activité est relativement grande, mais elle est faible dans les parties internes des racines. Une connaissance de la répartition de l'activité de la linamarase permettra peut-être de développer des méthodes plus efficaces pour éliminer la toxicité des produits du manioc. Le processus de dégradation des glucosides de la partie intérieure râpée des racines tubéreuses peut être considérablement accéléré par l'addition de feuilles ou d'écorce de racines tubéreuses, après quoi l'acide cyanhydrique peut être enlevé.

THIS paper is based on an extensive study of cassava toxicity which the author carried out in the Ivory Coast during 1965–70 (de Bruijn 1971).

In the last 15 years a great deal of attention has been paid to cyanogenesis in different plants. An understanding of this subject in cassava is of particular importance in view of the role played by this plant in both human and animal nutrition. The chemical pathway of cyanogenic glucosides is now well known. We know that cyanogenesis is connected with protein metabolism, that amino acids can serve as precursors of cyanogenic glucosides, and that there is a structural relationship between the cyanogenic glucoside and the precursor amino acid. In cassava, Nartey (1968) proved that valine and isoleucine can serve as precursors of the aglycone moieties of its cyanogenic glucosides linamarin and lotaustralin.

The cyanogenic glucoside content of the tuberous roots of cassava depends on many factors. The most important of these is the genetic constitution of the clone. However, since environmental conditions also have a very important influence, and since different clones react differently to varying ecological conditions, it is very difficult to make a reliable prediction of the cyanogenic glucoside content of the tuberous roots of a specific clone in a specific location.

We have not attempted to study the genetic background of the probably polygenic character of cassava cyanogenesis, but have paid particular attention to the influence of ecological conditions.

In an effort to obtain a better understanding of the behaviour of clones with different cyanogen levels we selected for our studies two clones having a rather low (about 50 $\mu$g HCN/g fresh weight), and two clones having a rather high (about 200 $\mu$g HCN/g fresh weight) cyanogenic glucoside concentration in the peeled tuberous roots.

As far as we know, no noncyanogenic cassava clones have yet been found.

## Distribution of the Glucoside

Results of a study on the distribution of the glucoside in the plant are presented in Table 1. As in other cyanogenic plants the glucoside concentration in the leaves decreases with age. In expanding leaves, the concentration in the leaf stalks is higher than that in the leaf blades, but in older leaves the reverse is true. In the bark of the leafless part of the stem, the glucoside concentration increases markedly from the top downwards. In the bark of tuberous roots, the concentration is much higher than in the inner part, this difference being relatively much more important in the less toxic clones than in the very toxic ones (Table 2).

From the standpoint of toxicity, clones are generally classified according to the glucoside content of the peeled tuberous roots, and it is often suggested that this classification is also valid for other parts of the plant. After analysing the glucoside concentration of the leaves and bark of

TABLE 1. Distribution of glucoside (*μg HCN/g fresh weight*) in different parts of plants of four clones.

| Part of plant | Clones | | | | |
|---|---|---|---|---|---|
| | Tabouca | A 13 | Ta 25 | 461 | Average |
| Leaf blades | | | | | |
| Very young, in expansion | 330 | 330 | 490 | 790 | 490 |
| Just full-grown | 420 | 340 | 570 | 1040 | 590 |
| Older | 250 | 210 | 320 | 730 | 380 |
| Leaf stalks | | | | | |
| Very young, in expansion | 400 | 750 | 770 | 940 | 720 |
| Just full-grown | 210 | 350 | 350 | 460 | 340 |
| Older | 120 | 110 | 170 | 180 | 150 |
| Stem bark | | | | | |
| Near oldest leaves | 270 | 350 | 550 | 1330 | 630 |
| At $\frac{2}{3}$ of leafless part | 90 | 230 | 330 | 580 | 310 |
| At $\frac{1}{3}$ of leafless part | 190 | 420 | 430 | 650 | 420 |
| Lowest part | 550 | 680 | 900 | 970 | 780 |
| Bark of cutting | 190 | 370 | 810 | 390 | 440 |
| Bark of tuberous roots | 400 | 540 | 890 | 730 | 640 |
| Inner part of tuberous roots | 36 | 55 | 210 | 240 | 140 |

TABLE 2. Average glucoside concentration (*μg HCN/g fresh weight*) in leaves, and in bark and inner part of tuberous roots, of less toxic and very toxic clones.

| | Tuberous roots | | |
|---|---|---|---|
| | Bark | Inner part | Leaves |
| Less toxic (avg 8 clones) | 690 | 73 | – |
| Very toxic (avg 8 clones) | 840 | 330 | – |
| Less toxic (avg 15 clones) | – | 60 | 770 |
| Very toxic (avg 15 clones) | – | 340 | 1040 |

tuberous roots, and of the inner part of these roots, from a large number of clones we found that this suggestion is not strictly correct. The glucoside concentration of the leaves and bark of tuberous roots of less toxic clones tends to be only slightly lower than that in the same organs of very toxic ones, although this is not so in the case of the glucoside concentration of the inner part of the tuberous roots.

It appears that the less toxic clones have a higher rate of degradation of the glucoside than do the very toxic ones, the rate of formation of the glucoside being more or less equal for both types of clones. Although the glucoside concentration may vary greatly between the tuberous roots of one plant, there is no correlation between glucoside concentration and tuber size.

There is also considerable variation in the distribution of the glucoside within a tuberous root. In almost every case the highest concentration is found at the proximal end of the root. In a horizontal section there is an increase in glucoside concentration from the centre outwards.

## Factors Influencing Cyanogenesis

In studying the influence of fertilizers, we found that nitrogen increased and potassium and farmyard manure decreased the glucoside content of leaves and roots. In general, the influence of phosphate, calcium, and magnesium was not important.

The supposition that glucoside concentration is positively correlated with the availability of amino acids in the plant may explain the influence of nitrogen and potassium, because manuring with nitrogen increases, and with potassium decreases,

the amino acid content in the leaves of various plant species (Ozbun 1965; Mengel and Helal 1968; Helal and Mengel 1968).

Serious drought increased glucoside content; short drought periods generally had little effect as the plant adapted by abscission of some leaves.

Somewhat surprisingly there seems to be a relationship between the glucoside content of a clone and its dry-matter content. We found that the glucoside concentration of the roots was negatively correlated with the dry-matter content of the leaves ($r = -0.33$) and also with that of the tuberous roots themselves ($r = -0.40$). Leaf glucoside concentration was also negatively correlated with the dry-matter content of the tuberous roots ($r = -0.34$) and with that of the leaves themselves ($r = -0.29$).

Shading young plants caused an increase in the glucoside concentration of their leaves and a decrease of that in the roots.

We did not find any relation between the glucoside content of tuberous roots and the age of the plant. We think it is more likely that differences in the glucoside content of tuberous roots at successive samplings are due to changing ecological conditions, rather than to changes in the age of the plant itself.

In Indonesia people believe that planting cuttings upside down will increase the toxicity of the tuberous roots of the resulting plants. Bolhuis (1939) did not find convincing evidence supporting this belief, nor did we in our experiments.

It is often said that there is a positive correlation between the productivity of a clone and its toxicity. Comparing 67 clones we did find a small positive correlation between the glucoside content of peeled tuberous roots and the amount of leaf ($r = 0.20$), stem ($r = 0.24$), and tuberous root ($r = 0.20$) per plant (significance level: for $P = 0.05$, $r = 0.24$).

## Possible Transport of Glucoside

The results of our studies on the distribution of the glucoside in the plant suggest there might be some transport of glucoside occurring within the plant.

Ringing of stems caused a considerable increase (more than 100%) of glucoside concentration in the bark above the incision, especially during the first few days. This accumulation was maintained for at least 2 months. Such an increase was not observed when leaves were eliminated. The effect of stem ringing, after 3 days, was more important in young plants (165%) than in older plants (65%). But we did not find an increase of the glucoside in the leaves above the incision.

Stem ringing caused a decrease in the glucoside content of the tuberous roots which fell by about 20% in 2 weeks.

Although these experiments indicate a transport and accumulation of the glucoside, it could be the precursors of the glucoside (e.g. amino acids) that are being transported. The use of radioisotopes would help to resolve this issue.

## Activity of the Enzyme Linamarase

Because the enzyme linamarase is of great importance for the breakdown of the glucoside we thought it necessary to study the activity of this enzyme in the plant. The results of a study of the distribution of linamarase in the plant are presented in Table 3.

The enzyme activity in the plant is highest in the very young expanding leaves and lowest in the lowest part of the stem bark and in the inner part of the tuberous roots. In the bark of the stem there is a marked decrease in activity from the top downwards. In the bark of the tuberous roots the activity is many times higher than in the inner part of these roots.

Very little is known about the role of linamarase in the plant. Our results do not indicate any evidence of a direct relationship between the concentration of enzyme in the plant and the level of glucoside. Linamarase activity in both the bark and the inner part of the tuberous roots of very toxic clones differed little from that found in less toxic clones.

A knowledge of the distribution of linamarase activity is important for an understanding of the process of eliminating the glucoside after harvesting. It also offers possibilities for developing more effective methods to eliminate toxicity of cassava products used for human and animal food.

## Elimination of the Glucoside

Tuberous roots of cassava, which contain a high level of glucoside, have to be specially treated

TABLE 3. Distribution of linamarase activity (*μg HCN liberated/g fresh weight per min*) in different parts of plants of four clones.

| Part of plant | Clones | | | | |
|---|---|---|---|---|---|
| | Tabouca | A 13 | Ta 25 | 461 | Average |
| Leaf blades | | | | | |
| Very young, in expansion | 450 | 1000 | 600 | 850 | 730 |
| Just full-grown | 400 | 600 | 100 | 100 | 300 |
| Older | 200 | 150 | 10 | 40 | 100 |
| Leaf stalks | | | | | |
| Very young, in expansion | 650 | 1150 | 350 | 800 | 740 |
| Just full-grown | 200 | 550 | 300 | 400 | 360 |
| Older | 250 | 600 | 300 | 350 | 380 |
| Stem bark | | | | | |
| Near oldest leaves | 160 | 170 | 130 | 130 | 150 |
| At $\frac{2}{3}$ of leafless part | 140 | 110 | 20 | 70 | 90 |
| At $\frac{1}{3}$ of leafless part | 80 | 160 | 30 | 45 | 80 |
| Lowest part | 0 | 15 | 0 | 10 | 6 |
| Bark of cutting | 10 | 120 | 0 | 15 | 35 |
| Bark of tuberous roots | 140 | 480 | 160 | 280 | 270 |
| Inner part of tuberous roots | 9 | 13 | 6 | 7 | 9 |

to eliminate the toxic substances before they can be eaten. Many treatments for eliminating toxicity are known, but confusion still exists about their adequacy. Though cassava consumers generally know how to get rid of the toxic substance, intoxication accidents do still occur.

Treatments to reduce the danger of intoxication can be divided into two groups:

1. The glucoside is eliminated. This elimination can be direct (e.g. soaking) or by enzymic breakdown.

2. The glucoside is only partly eliminated, if at all, but the enzyme is broken down (e.g. heating). Combinations of both groups also exist.

The best way to obtain a safe product is to break down the glucoside by the enzyme and then to eliminate the HCN by drying or heating. The speed and adequacy of this process depends on the intensity of the contact between the glucoside and the enzyme, thus on the rate of grating of the tuberous roots, and, self-evidently, on the enzyme activity. In the leaves and in the bark of tuberous roots linamarase activity is so high that in a very short period after crushing or grating all the glucoside is broken down and the HCN can be driven off. But in the inner part of the tuberous roots linamarase activity is very low and it may take a long time before all the glucoside has been broken down. To eliminate toxicity, tuberous roots are often grated and fermented for one or more days. However, according to Adriaens (1955) this decreases the quality of the product.

We found that it was possible to considerably accelerate the process of breaking down the glucoside of the grated inner part of the tuberous roots by the addition of juice prepared from the leaves or bark of tuberous roots. This caused all of the glucoside present to be broken down within 1 h. Thus, when preparing food for animal consumption, it was sufficient to grate tuberous roots as a whole, including the bark. For human consumption this method might render the taste less pleasant. More research is necessary in this area.

The addition of leaves or of bark of tuberous roots to preparations of food from the inner part of the roots is also important from a nutritional point of view, since the protein content of cassava leaves is very high, and the nutritive value of the

root bark is also much higher than that of the inner part of the tuberous roots (Barrios and Bressani 1967).

When the glucoside is not eliminated, but only the enzyme is broken down, questions arise about the safety of the product. A good example is cooked tuberous roots. It has often been stated that cooking is a safe method for the elimination of toxicity of cassava roots. In fact, cooking destroys the enzyme at 72°C (Joachim and Panditteseker 1944), but not the glucoside. When the tuberous roots are cooked, the glucoside cannot get out of the starchy product and we found that up to 90% of the original quantity may remain. The safety of eating this product depends on the quantity of glucoside present, and of the quantity that can be safely consumed by man. According to Montgomery (1965) it has never been proved that the glucoside itself is toxic to man. Part of the glucoside consumed will be broken down by stomach acids. It is a moot point what part of this glucoside will be broken down and what conditions are important for this to take place.

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# The Genetics of Cyanogenesis

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**Abstract** Part of the variation in the levels of hydrogen cyanide (HCN) production, which exists between cyanogenic plants, is genetically controlled in *Sorghum* species, *Lotus* species, and *Trifolium repens*. In *Sorghum* no discrete class for HCN production can be recognised in a population of individuals showing variation in levels of HCN. The genetic control of HCN production in *Sorghum* species is considered multigenic. In *Lotus* and *Trifolium* another form of variation exists in which discrete classes can be recognised. In *Trifolium repens* the locus *Ac* controls the presence or absence of the cyanogenic glucosides, linamarin and lotaustralin, and the locus *Li* controls the presence or absence of the enzyme which hydrolyses these glucosides. Plants possessing only nonfunctional alleles of at least one of the loci are acyanogenic. Biochemical and genetical studies on the cyanogenic system of *Trifolium repens* have shown the presence of inherited variation in the level of hydrolytic enzyme present in plants possessing functional *Li* alleles. Using this variation, together with immunological studies, it has not been possible, as yet, to demonstrate that the *Li* locus is a structural gene for this enzyme. Comparison of the linamarin biosynthetic pathway between *Trifolium* plants which produce the cyanoglucosides and plants which do not, has suggested that the *Ac* locus may control more than one step in this biosynthetic pathway.

**Résumé** Une partie des variations du niveau d'acide cyanhydrique (HCN) observées chez certaines plantes cyanogènes sont sous contrôle génétique chez les espèces de *Sorghum*, de *Lotus* et chez *Trifolium repens*. Dans une population d'individus du genre *Sorghum* montrant des variations de niveau de HCN, on ne peut déceler de classes discontinues quant à la production de HCN. On est généralement d'avis que la production de HCN par les espèces de *Sorghum* est contrôlée par plusieurs gènes. Par ailleurs, il existe chez *Lotus* et *Trifolium* une autre forme de variations qui peuvent être groupées en classes discontinues. Chez *Trifolium repens*, le locus *Ac* contrôle la présence ou l'absence des glucosides cyanogènes, linamarine et lotaustraline, alors que le locus *Li* contrôle la présence ou l'absence de l'enzyme hydrolysant ces glucosides. Les plantes qui ne possèdent que des allèles non fonctionnels d'au moins un des loci sont acyogènes. Des études biochimiques et génétiques sur le système cyanogène de *Trifolium repens* démontrent une variation héréditaire du niveau de l'enzyme hydrolytique présent chez les plantes possédant des allèles *Li* fonctionnels. En se basant sur cette variation et sur des études immunologiques, il a été impossible, à venir jusqu'ici, de démontrer que le locus *Li* est un gène structural contrôlant cet enzyme. Une comparaison de la voie biosynthétique de la linamarine entre les plants de *Trifolium* qui produisent les cyanoglucosides et ceux qui ne les produisent pas suggère que le locus *Ac* peut contrôler plus d'une étape de cette voie biosynthétique.

ALL the cyanogenic plant species which have been investigated in any detail show variation in the amount of hydrocyanic acid (HCN) produced. This variation reflects variation in both the production of cyanoglucosides themselves and the enzymes which degrade them. Variation occurs within individual plants, or genotypes, depending on the tissue analysed, the age of the tissue, and the cultural conditions of the plant (Sinha and Nair 1968; Gillingham et al. 1969; Roger and Frykolm 1937). Variation in HCN production also occurs between individual plants, and it is this variation which has been studied by geneticists and plant breeders. Since individual plants show variation depending on cultural conditions, part of the variation between plants must be due to environmental differences. However, in those species studied, some of the interplant variation is due to the genotype of the plants and is therefore genetically controlled.

The grass species *Sorghum sudanense* (sudangrass) and *S. bicolor* produce the cyanoglucoside, dhurrin. This glucoside is broken down by two enzymes, $\beta$-glucosidase and oxynitrilase, to give glucose, *p*-hydroxybenzaldehyde, and HCN. In order for a plant to produce HCN it must be able to synthesise dhurrin and the enzymes which degrade dhurrin. Both these grasses and the hybrid, sorghum-sudangrass, are used as animal feed and plant breeders have been working for a number of years to produce low-HCN strains.

The variation in HCN content of these grasses is continuous, that is it is not possible to recognise any discrete class of individuals within a variable population of plants. A number of studies of the inheritance of HCN production in sudangrass and sorghum-sudangrass hybrids have been made, without the same results (Nass 1972). Authors differ in their conclusions about the number of genes involved and the dominance relationships of these genes. For example, Snyder (1950) concluded that in sudangrass there was one pair of genes determining high versus low cyanide content and that low HCN production was dominant over high HCN production. Barnett and Caviness (1968), on the other hand, showed that in sorghum-sudangrass hybrids the inheritance of HCN production was multigenic, with high HCN production partially dominant to low HCN production.

There has been some interest in finding an association between low HCN production and other simply inherited characters in sorghum. In sorghum-sudangrass hybrids, Carlson (1958) found low HCN production associated with the following loci: green striped-1, golden-2, yellow leaf tip, and single seeded spikelets. Pleiotropy seemed a more likely explanation of this phenomenon than linkage.

In three plant species, *Trifolium repens* (white clover), *Lotus tenuis*, and *L. corniculatus* (birdsfoot trefoil), another form of variation exists in which discrete classes can be recognised, namely plants which produce HCN when damaged and plants which produce no HCN when damaged. Continuous variation in HCN production also exists in those plants capable of releasing HCN (Jones 1962; Corkill 1942), so that these plants may also be arbitrarily classified high, low, or intermediate for HCN production. These three species produce two structurally related cyanoglucosides, linamarin and lotaustralin, and HCN release occurs when these are hydrolysed by the enzyme, linamarase, to produce glucose and an unstable aglycone, which undergoes further decomposition to give HCN and either acetone or methylethyl ketone.

In white clover the discrete form of variation is controlled by two genes (Corkill 1942). The presence or absence of both the glucosides is governed by alleles of a single gene, designated *Ac*, whereas the presence or absence of the enzyme linamarase is governed by alleles of another, independently inherited, gene (*Li*). Only plants which possess dominant functional alleles of both genes liberate HCN when damaged. However, the presence of a functional allele of only one of these genes may be shown by adding either a glucoside or enzyme extract to the test material. The inheritance of cyanogenesis is diploid in white clover. The genetic system in *Lotus* is very similar except that *L. corniculatus* is an autotetraploid and the inheritance of HCN production is tetrasomic.

Biochemical aspects of plant breeding have gained increasing prominence over the past few years, although our understanding of the genetic control of metabolism in plants is still rudimentary. The difficulties of studying gene action in higher plants have been well documented (Nelson 1967), but these studies are essential if we are to avoid the obvious danger of extrapolating information gained from microorganisms without the means of testing such hypotheses. My interest in cyanogenesis has arisen as the result of an attempt to use the cyanogenic polymorphism of white

TABLE 1. Specific activities (as *micromoles substrate hydrolysed per milligram of protein in 10 min*) of extracts of white clover plants.

| Plant | Genotype | No. samples | Mean specific activity (±SE) | | |
|---|---|---|---|---|---|
| | | | Linamarin–lotaustralin | *p*-Nitrophenyl β-D-glucoside | *p*-Nitrophenyl β-D-galactoside |
| CS | *LiLi* | 3 | 2.58 ± 0.23 | 3.44 ± 0.16 | 2.07 ± 0.10 |
| C2 | *LiLi* | 6 | 2.35 ± 0.16 | 3.55 ± 0.18 | 1.94 ± 0.08 |
| C3 | *Lili* | 3 | 0.60 ± 0.02 | 1.18 ± 0.03 | 0.65 ± 0.04 |
| C4 | *Lili* | 3 | 0.93 ± 0.04 | 1.60 ± 0.01 | 0.86 ± 0.03 |
| C5 | *Lili* | 3 | 0.64 ± 0.04 | 1.14 ± 0.04 | 0.64 ± 0.02 |
| C7 | *Lili* | 3 | 1.20 ± 0.06 | 1.99 ± 0.06 | 1.07 ± 0.04 |
| C10 | *Lili* | 3 | 0.87 ± 0.01 | 1.52 ± 0.03 | 0.87 ± 0.02 |
| C11 | *Lili* | 3 | 0.20 ± 0.01 | 0.57 ± 0.02 | 0.37 ± 0.03 |
| C13 | *Lili* | 3 | 0.89 ± 0.02 | 1.69 ± 0.03 | 0.91 ± 0.02 |
| C14 | *Lili* | 3 | 1.31 ± 0.03 | 2.21 ± 0.03 | 1.26 ± 0.05 |
| D4 | *lili* | 3 | 0 | 0.16 ± 0.01 | 0.19 ± 0.003 |
| D9 | *lili* | 6 | 0 | 0.31 ± 0.01 | 0.21 ± 0.01 |

clover in a detailed study of gene action in higher plants. Specifically, we have attempted to define the nature of the *Ac* and the *Li* loci in white clover.

## Experimental

The *Li* locus controlling linamarase activity was the first choice because of the direct relationship between gene and enzyme, established in micro-organisms. The *Li* locus may represent the gene specifying the structural information for the enzyme, linamarase, or it may control the amount of enzyme synthesised in the plant. Mixing experiments have shown that the loss of linamarase activity in plants homozygous for the nonfunctional allele is not due to the production of enzyme inhibitors in this genotype (Hughes 1970). The system is slightly complicated, because the *Li* locus controls a number of enzyme activities and it is not clear how many distinct enzyme proteins are involved in these activities.

Two approaches, one genetic the other immunological, have been used in this study. Immunological studies provided no evidence for a structural gene (Hughes and Maher 1973) since no inactive protein, immunologically related to the normal enzyme (cross-reacting material), was found in plants containing only nonfunctional alleles (*lili*). This is perhaps surprising because *lili* plants have some residual enzyme activity and the antigen–antibody complex of the normal enzyme (from *LiLi* plants) retains enzyme activity.

Table 1 (Maher and Hughes 1973) shows the mean specific activity of extracts from several white clover plants measured against three substrates. This table shows that the *lili* genotype having two nonfunctional alleles has no linamarase activity but has low activity towards *p*-nitrophenyl β-D-glucoside (PNPG) and *p*-nitrophenyl β-D-glucoside (PNPGAL). The lack of dominance of functional *Li* alleles is also indicated in Table 1, since the plants (CS and C2) homozygous for functional alleles have higher enzyme activities compared with the heterozygous plants. (This was confirmed by further breeding work.)

The rationale of the approach was as follows: the stability of enzymes to heat and certain inhibitors is a character which depends on the structure of enzyme protein. Differences in these characters may therefore be taken as evidence of differences in protein structure. Crosses are made between a plant possessing an altered linamarase and being heterozygous at the *Li* locus and plants possessing only recessive, nonfunctional alleles at the *Li* locus. The progeny from these crosses are scored for the presence of linamarase and for the properties of the enzyme. If the *Li* locus specifies the structure of linamarase, all individuals in the progeny containing a functional *Li* allele will also possess an enzyme having the modified characters of the heterozygous parent.

One of the heterozygous plants (C11), shown in Table 1, differed markedly from the other heterozygous plants in having a much lower specific

TABLE 2. Specific activities (*as micromoles substrate hydrolysed per milligram of protein in 10 min*) of extracts of the progeny from crosses involving C7, C11, and C14.

| Parents | Genotype of progeny | No. progeny analyzed | Mean specific activity (±SE) | | |
|---|---|---|---|---|---|
| | | | Linamarin–lotaustralin | *p*-Nitrophenyl β-D-glucoside | *p*-Nitrophenyl β-D-galactoside |
| C7 × D4 | *Lili* | 20 | 1.52 ± 0.06 | 2.23 ± 0.06 | 1.35 ± 0.04 |
| | *lili* | 20 | 0 | 0.27 ± 0.01 | 0.18 ± 0.01 |
| C7 × D9 | *Lili* | 20 | 1.24 ± 0.05 | 1.89 ± 0.05 | 1.12 ± 0.03 |
| | *lili* | 10 | 0 | 0.27 ± 0.01 | 0.18 ± 0.01 |
| C11 × D4 | *Lili* | 18 | 0.22 ± 0.01 | 0.51 ± 0.03 | 0.32 ± 0.002 |
| | *lili* | 10 | 0 | 0.15 ± 0.01 | 0.17 ± 0.01 |
| C11 × D9 | *Lili* | 18 | 0.29 ± 0.01 | 0.65 ± 0.02 | 0.40 ± 0.01 |
| | *lili* | 10 | 0 | 0.22 ± 0.01 | 0.17 ± 0.01 |
| C14 × D4 | *Lili* | 16 | 1.22 ± 0.05 | 1.84 ± 0.06 | 1.08 ± 0.04 |
| | *lili* | 9 | 0 | 0.24 ± 0.02 | 0.17 ± 0.02 |
| C14 × D9 | *Lili* | 18 | 1.21 ± 0.12 | 1.78 ± 0.07 | 1.04 ± 0.04 |
| | *lili* | 20 | 0 | 0.28 ± 0.01 | 0.17 ± 0.01 |

activity toward all three substrates. The effect of individual *Li* and *li* alleles was made by making crosses between C11 and the two *lili* plants given in Table 1. Similar crosses were made between two other heterozygous plants (C7 and C14) and the *lili* plants. Measurements of enzyme activity were then made on the progeny of these crosses, and the results are given in Table 2. The levels of activity of all the heterozygous progeny from the C11 crosses were distinguishable from the activities of all the heterozygous progeny from the remaining four crosses. This is shown in Table 2 by the difference in mean specific activities and the low standard error of means for these crosses. This analysis of the *Lili* heterozygous progeny supports the conclusion that the functional allele of C11 is controlling the activity of either a reduced level of, or a low activity form of, the enzyme(s) responsible for the hydrolysis of linamarin, lotaustralin, PNPG, and PNPGAL.

Three characters were investigated to test for the presence of an altered enzyme protein in the low activity plant C11 and two *lili* plants. No evidence for an altered enzyme was found in C11, but the plants (D4 and D9) which contained only non-functional alleles were shown to possess a different β-glucosidase. However, this low-activity enzyme may also exist in plants having the functional alleles, where its presence would be masked by the more active linamarase. Subsequent work (Hughes and Ayre unpublished data) on cultured cells of white clover (which are not cyanogenic) has demonstrated this enzyme in *LiLi* genotypes, and so this work also provides no evidence for a structural gene at the *Li* locus.

## Discussion

The function of the locus (*Ac*) controlling cyanoglucoside production has been investigated (Hughes and Conn unpublished data) in a study designed to determine which step in the biosynthesis of linamarin and lotaustralin is affected by this gene. Figure 1 represents the proposed pathway for the biosynthesis of linamarin. The glucosyltransferase enzyme, which catalyses the final step (4) has been purified from linen flax (Hahlbrock and Conn 1970) but the isolation of the other enzymes involved has yet to be achieved and this meant that the direct measurement of enzyme activity could not be made. The presence of *N*-hydroxyvaline in this pathway has not been established and in this paper the conversion of valine to isobutyraldoxime is considered as a single step. Since Hahlbrock and Conn (1971) have shown that the biosynthesis of both linamarin and lotaustralin is catalysed by the same enzymes the study was limited to a comparison of the biosynthesis of linamarin between plants which contain the cyanoglucosides and plants which do not.

Table 3 shows the results of feeding U-$^{14}$C valine to shoots of white clover plants. The plants S100/1 and S100/10 produced cyanoglucosides but no linamarase, and the plants DWW/1 and DWW/3

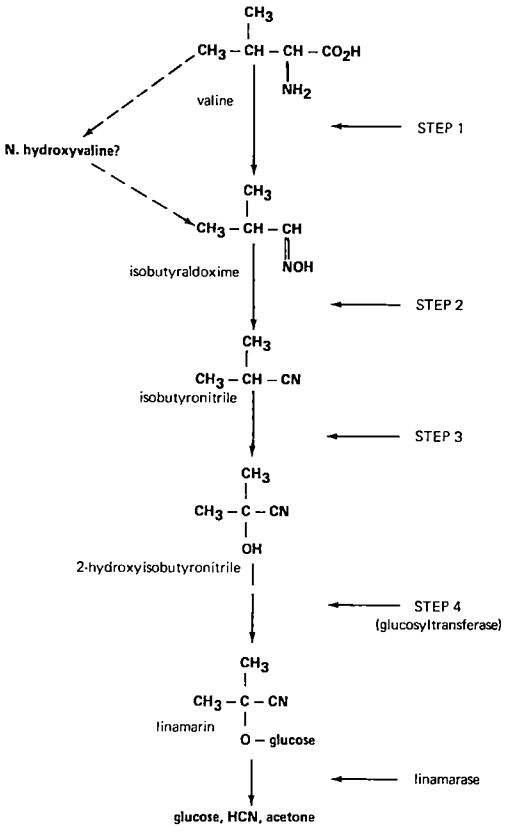


FIG. 1. The biosynthesis of linamarin (from Tapper and Butler 1971).

TABLE 3. Incorporation of $^{14}$C-valine into linamarin in white clover.

| Plant | HCN content[a] | Fresh weight (*g*) | U-$^{14}$C valine administered (*μci*) | Converted to linamarin (%) |
|---|---|---|---|---|
| S100/1 | 2.60 | 0.6 | 0.92 | 6.6 |
| S100/10 | 2.44 | 0.9 | 0.92 | 6.5 |
| DWW/1 | 0 | 0.7 | 0.92 | 0 |
| DWW/3 | 0 | 0.5 | 0.92 | 0 |

[a]Expressed as μmoles/100 mg fresh weight, first expanded leaf.

TABLE 4. Incorporation of $^{14}$C-isobutyraldoxime into linamarin in white clover.

| Plant | HCN content[a] | Fresh weight (*g*) | U-$^{14}$C isobutyraldoxime administered (*μci*) | Converted to linamarin (%) |
|---|---|---|---|---|
| S100/1 | 2.60 | 1.0 | 0.90 | 2.4 |
| S100/1 | 2.60 | 0.7 | 0.90 | 2.5 |
| S100/10 | 2.44 | 0.9 | 0.90 | 2.0 |
| S100/10 | 2.44 | 0.6 | 0.90 | 2.6 |
| DWW/1 | 0 | 0.6 | 0.90 | 0 |
| DWW/1 | 0 | 1.0 | 0.90 | 0 |
| DWW/3 | 0 | 0.7 | 0.90 | 0 |
| DWW/3 | 0 | 0.7 | 0.90 | 0 |

[a]Expressed as μmoles/100 mg fresh weight, first expanded leaf.

TABLE 5. Incorporation of $^{14}$C-valine into linamarin in white clover shoots treated with isobutyraldoxime.

| Plant | HCN content[a] | Fresh weight (*g*) | Isobutyraldoxime administered (*μmoles*) | U-$^{14}$C valine administered (*μci*) | Converted to linamarin (%) | Trapped as isobutyraldoxime (%) |
|---|---|---|---|---|---|---|
| S100/1 | 2.60 | 1.5 | 43.2 | 3.68 | 2.9 | 0.3 |
| S100/10 | 2.44 | 0.6 | 21.6 | 1.84 | 3.5 | 0.7 |
| DWW/1 | 0 | 1.3 | 43.2 | 3.68 | 0 | 0 |
| DWW/3 | 0 | 0.7 | 21.6 | 1.84 | 0 | 0 |

[a]Expressed as μmoles/100 mg fresh weight, first expanded leaf.

contained neither cyanoglucoside nor the hydrolytic enzyme. It can be seen that the conversion of $^{14}$C valine into linamarin has only occurred in those plants normally capable of synthesising cyanoglucosides. Table 4 shows the results of feeding U-$^{14}$C isobutyraldoxime to the same plants. Again, the incorporation of radioactivity into linamarin has only occurred in those plants (S100/1 and S100/10) normally capable of linamarin synthesis. This means that at least one step after the production of isobutyraldoxime is missing in plants DWW/3 and DWW/1.

To test for the presence of step-one function (isobutyraldoxime synthesis) in cyanoglucoside negative plants, $^{14}$C-labelled valine was fed to the same four plants in the presence of excess cold isobutyraldoxime. The results of this experiment are shown in Table 5, where it can be seen that in

the cyanoglucoside-positive plants (S100/1 and S100/10) about 0.5% of the radioactivity fed as valine has been "trapped" by the cold isobutyraldoxime thus demonstrating the function of step one in these plants. In the cyanoglucoside-negative plants (DWW/1 and DWW/3), no measurable radioactivity was found in the isobutyraldoxime fraction. This result means that white clover plants unable to synthesise linamarin lack at least two steps in the biosynthetic pathway shown in Fig. 1.

This conclusion means either that the single gene nature of the *Ac* locus must be questioned or that this locus may not represent a gene specifying structural information for protein synthesis. Detailed studies of both the genes controlling cyanogenesis in white clover have therefore tended to suggest that they represent control elements rather than structural genes.

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# Cyanogenic Glycosides: Their Occurrence, Biosynthesis, and Function

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CONN, ERIC E. 1973. Cyanogenic glycosides: their occurrence, biosynthesis, and function, p. 55–63. *In* Chronic cassava toxicity: proceedings of an interdisciplinary workshop, London, England, 29–30 January 1973. Int. Develop. Res. Centre Monogr. IDRC-010e.

**Abstract** Cyanogenic glycosides are widely distributed among plants and in two classes of animals (Myripoda and Insecta). The structure and distribution of some cyanogenic glycosides are discussed, in particular the structure of linamarin and lotaustralin which occur in cassava (*Manihot* spp.). The biosynthesis and functions of these compounds are discussed, as well as their possible role in the etiology of tropical ataxic neuropathy and goitre.

**Résumé** Les glycosides cyanogènes sont largement répandus parmi les plantes et dans deux classes d'animaux (Myripoda et Insecta). L'auteur examine la structure et la distribution de quelques glycosides cyanogènes, plus particulièrement la structure de la linamarine et de la lotaustraline, qui se trouvent dans le manioc (*Manihot* sp.). Il discute de la biosynthèse et des fonctions de ces composés, de même que leur rôle possible dans l'étiologie de la neuropathie ataxique et du goitre dans les régions tropicales.

THE cyanogenic glycosides may be defined chemically as glycosides of α-hydroxynitriles (cyanohydrins). They have a wide distribution among the higher plants but also occur in some ferns, and two classes of animals (Myripoda and Insecta). Cyanogenic glycosides will release prussic or hydrocyanic acid (HCN) upon treatment with dilute acids, usually at elevated temperatures. However, the phenomenon of "cyanogenesis," the production of HCN from these compounds, is usually due to the action of enzymes present in the tissues of cyanophoric plants. The action of the enzymes is initiated by crushing or otherwise destroying the cellular structure of the plant.

The present scientific interest in these compounds arises from at least three different areas. Firstly, the toxicity of many cyanophoric plants can be directly attributed to their ability to produce a high level of HCN, a potent inhibitor of cellular respiration. The tubers of cassava (*Manihot* spp.) and the leaves of sorghum and cherry laurel can produce from 25 to 250 mg HCN/100 g of fresh tissue. These and other plants have been responsible for many cases of acute cyanide poisoning of animals including man (Kingsbury 1964; Montgomery 1969). Secondly, the unusual chemical structure of cyanogenic glycosides has attracted the interest of organic chemists for more than a century. More recently, biochemists have concentrated on the metabolism of these compounds in the plants in which they are found. Recent reviews emphasizing both the chemical features (Eyjolfsson 1970) and the metabolism of the cyanogenic glycosides (Conn 1969; Conn and Butler 1969; Conn 1973) have appeared. Thirdly, considerable interest has centred on the possible role of two of these compounds in the etiology of tropical ataxic neuropathy and goitre (Montgomery 1969). Indeed, it is this subject which has provided the impetus for this meeting.

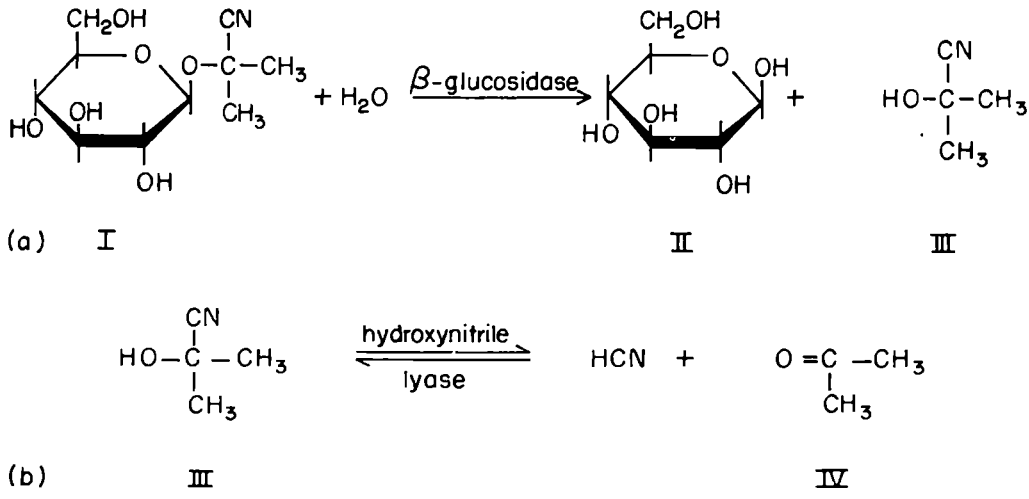


FIG. 1. The mechanism of enzymatic decomposition of linamarin.

## Structure and Distribution

Figure 1 shows the structure of linamarin (I), one of the two cyanogenic glucosides that occur in cassava. Also represented is the process by which HCN can be produced in this plant from linamarin. In step *a*, the β-glucosidic bond linking β-(D)-glucose(II) to 2-hydroxyisobutyronitrile (acetone cyanohydrin) (III) is hydrolyzed by the endogenous β-glucosidase (linamarase) to form those two compounds. In step *b*, the hydroxynitrile dissociates to form acetone (IV) and HCN. While this process can and does readily occur non-enzymically, enzymes catalyzing this type of reaction (hydroxynitrile lyases) are known and have been studied in *Sorghum vulgare* (Seely et al. 1966) and the Rosaceae (Gerstner et al. 1968). The presence of such an enzyme in higher plants such as cassava that contain linamarin (and lotaustralin) may therefore be predicted.

The process represented in Fig. 1 is the one usually employed to determine if a specific plant is cyanogenic. The HCN released upon crushing or otherwise destroying the plant tissue can be detected by sensitive, qualitative, colorimetric tests; Eyjolfsson (1970) may be consulted for descriptions for three such tests. In addition, the recognition of the characteristic odor of acetone (from linamarin) or benzaldehyde (from amygdalin, prunasin, sambunigrin, and vicianin) has served to indicate tentatively the presence of cyanogenic glycosides in many species.

It should perhaps be noted that the production of HCN according to Fig. 1 is dependent not only on the presence of the parent cyanogenic glycoside but also on the enzymes that accomplish its decomposition. It is well established that varieties of *Lotus corniculatus* and *Trifolium repens* may lack either or both of these factors and, as discussed elsewhere, these capabilities are under genetic control. There are indications in the literature that other plants such as certain acacias (Finnemore and Gledhill 1928) may produce the glucoside but lack the enzyme(s) which degrade it. The fact that a single species may possess both bitter and sweet varieties may be an indication that cyanogenesis is under genetic control in that species (Jones 1972).

Table 1 lists 5 of the 20 known cyanogenic glucosides, some of the plants in which they occur, and the products formed on hydrolysis. These cyanogens have been chosen to illustrate several points regarding the chemistry and distribution of these compounds. First, in the case of linamarin and lotaustralin, it may be pointed out that, with one exception, these two cyanogens always appear together in the same species. Butler (1965) examined 20 species reported to contain linamarin or lotaustralin and, with the exception of *Hevea brasiliensis*, showed that both compounds were present, albeit in widely varying ratios. Thus in cassava, linamarin accounted for 96% of the cyanogenic material and lotaustralin therefore only 4%. On the other hand, the two compounds

TABLE 1. Some cyanogenic glycosides.

| Glycoside | Some plant sources | Hydrolysis products |
|---|---|---|
| Linamarin | *Dimorphotheca berberiae* (Compositae); several *Manihot* sp., *Hevea brasiliensis, Cnidoscolus texanus* (Euphorbiaceae); *Linum* sp. (Linaceae); *Papaver nudicaule* (Papaveraceae); many sp. of *Lotus, Phaseolus lunatus* and *Trifolium repens* (Papilionaceae) | D-glucose + HCN + acetone |
| Lotaustralin | Occurs with linamarin | D-glucose + HCN + 2-Butanone |
| Prunasin | *Eremophila maculata* (Myoporaceae); *Eucalyptus cladocalyx* (Myrtaceae); *Cystopteris fragilis, Pteridium aquilinum* (Polypodiaceae); species of *Cydonia, Eriobotrya, Prunus, Pyrus* and other genera (Rosaceae); *Jamesia americana* (Saxifragaceae); *Linaria* sp. (Scrophulariaceae) | D-glucose + HCN + Benzaldehyde |
| Dhurrin | *Sorghum vulgare, Bambusa arundinacea, Zea mays* (Gramineae) | D-glucose + HCN + *p*-Hydroxybenzaldehyde |
| Zierin | *Zieria laevigata* (Rutaceae) | D-glucose + HCN + *m*-Hydroxybenzaldehyde |

are present in the ratio of 55:45 in linen flax, a plant in which the biosynthesis of these compounds has been thoroughly examined (Tapper and Butler 1971). The simultaneous occurrence of these two compounds in a single species is attributed to the existence of a set of biosynthetic enzymes that can act on both valine and isoleucine converting these amino acids to linamarin and lotaustralin respectively (Hahlbrock and Conn 1971).

Another noteworthy feature of linamarin and lotaustralin is their relatively broad distribution in the plant kingdom, having been demonstrated in the following families of higher plants: Compositae, Euphorbiaceae, Linaceae, Papaveraceae, and Papilionaceae (Eyjolfsson 1970; Hegnauer 1971). A similar wide distribution has been observed for prunasin in six families (Myoporaceae, Myrtaceae, Polypodiaceae, Rosaceae, Saxifragaceae, and Scrophulariaceae). On the other hand, sambunigrin, vicianin, and amygdalin, which are closely related in chemical structure to prunasin, have been reported in only three (Caprifoliaceae, Mimosaceae, and Oleaceae), two (Polypodiaceae and Papilionaceae), and one (Rosaceae) families respectively. With the exception of these compounds, the more common distribution pattern is that a particular cyanogenic compound will occur in only one or two families (e.g. dhurrin only in the Gramineae) and in several instances the cyanogen will have been unequivocally demonstrated and characterized only in a single species (e.g. zierin in *Zieria laevigata*, family Rutaceae).

Conversely, it is generally true that, with few exceptions, only one characteristic glycoside will occur in a given family. Thus the Gramineae appear to contain only dhurrin and the Compositae only linamarin (and lotaustralin). However, the Polypodiaceae contain both prunasin (in *Cystopteris fragilis* Bernli) and vicianin (in three species of the genus *Davallia*). It can be pointed out that prunasin and vicianin are chemically similar, especially when one considers the biosynthetic origin of their aglycones from phenylalanine. Similarly the Rosaceae contain both amygdalin and prunasin. In this family these two closely related compounds are frequently observed in the same species, the amygdalin occurring in the seeds and prunasin in the leaves or other vegetative tissue. One intriguing family is the Mimosaceae where a single genus *Acacia* contains two quite dissimilar cyanogens. Sambunigrin, which gives rise to benzaldehyde on hydrolysis, has been isolated from *A. glaucescens*, an Australian species (Finnemore and Cox 1928) while acacipetalin possessing an aliphatic aglycone has been isolated from a South African species *A. sieberiana* (Rimington 1935). In like manner, the family Papilionaceae contains vicianin in the genus *Vivia* while linamarin (and lotaustralin) occur in the genera *Lotus*, *Phaseolus*, and *Trifolium*.

The preceding remarks on the occurrence of cyanogenic glycosides needs to be considered in the following context. The task of classifying a plant as cyanogenic is fraught with uncertainty. For example, the quantity of HCN that will be released for a given amount of plant tissue is extremely variable ranging from trace amounts to as much as 500 mg from 100 g (fresh weight) of leaves of *Nandina domestica* (Abrol et al. 1966). The part of the plant containing the cyanogen varies, and should therefore be examined in any careful test. Thus, young vigorously growing tissue (leaves, hypocotyls) frequently contain the cyanogens but they are also found in seeds and stems (e.g. in the Rosaceae) and in tubers (in cassava). Finally, the amount of cyanogen in a given plant, like the content of many other secondary plant products (Fluck 1963), may vary greatly with soil, climate, and the geographical location as well as the age of the plant (Seifert 1955). Nevertheless, the number of plants known to produce HCN approaches 1000 species representing more than 70 families and 250 genera (see Hegnauer 1971 for general review). In spite of this, the number of species in which known cyanogenic compounds have been carefully identified number fewer than 50 species representing only 20 families. There obviously is a great need to identify the cyanogenic substance(s) in many of the remaining families to provide information of maximum value to the chemotaxonomist who would use these compounds as a tool.

Other common structural features of the cyanogenic glucosides can be recognized in part from information given in Table 1. For example, the sugar produced from a majority of the glycosides on hydrolysis is D-glucose. Moreover, where carefully determined, the glucose is present in the pyranosyl form with the $\beta$-configuration. The majority of the cyanogens, therefore, are *O*-$\beta$-(D)-glucopyranosides. The exceptions are amygdalin, vicianin, and lucumin (Eyjolfsson 1970, 1971) which are disaccharides that have a second sugar (glucose, arabinose, and xylose respectively) linked to the glucose that is bound in turn to the aglycone.

Table 1 also indicates that either an aldehyde or ketone is formed on hydrolysis of cyanogenic glycosides. These compounds are produced when the $\alpha$-hydroxynitrile that constitutes the aglycone of the glycoside dissociates to form HCN. Indeed the chemical structure of the aglycone has been the basis on which Robinson (1930), Dilleman (1958), and Eyjolfsson (1970) have organized their classification of these compounds.

With the recent work on the metabolism of the cyanogenic glycosides, it is also possible to classify these compounds on the basis of the biosynthetic origin of their aglycones (Conn 1973). Thus, the aglycones of 13 of the 20 known glycosides are formed, or may be assumed to be formed, from five proteinaceous amino acids. These are the three branch-chain amino acids, valine, isoleucine, and leucine, and the aromatic amino acids phenylalanine and tyrosine. The aglycones of the other seven cyanogens do not appear to be formed, at least directly, from proteinaceous amino acids. However, Conn (1973 in press) suggests that five of these could arise from L-2-cyclopentene-1-glycine, an amino acid which has not yet been shown to occur naturally. This method of classification of the cyanogens does not differ in principle from that used earlier, but does serve to raise certain questions. Why, for example, should some but not all of the cyanogenic glycosides be derived from protein amino acids? Then too, why should only the five amino acids cited serve as precursors of the aglycones of the cyanogens? Does this suggest that one will find the other proteinaceous amino acids serving as precursors when the structures of the cyanogenic compounds in sufficiently diverse plant material are examined?

## Biosynthesis

The biosynthesis of the cyanogenic glycosides has been recently reviewed (Conn 1969, 1973). Research in several laboratories has demonstrated a precursor–product relationship between four proteinaceous amino acids and the aglycones of several cyanogenic glycosides. This relationship is illustrated in Fig. 2 for four of the glycosides. The evidence consisted of feeding these amino acids labelled in one or more carbon atoms with $^{14}C$ to cyanogenic plants (or parts thereof) and measuring the incorporation of isotope into the aglycone of the glycoside after a period of metabolism.

Taking dhurrin as a specific example (Fig. 3), work in several laboratories has shown that the $\alpha$-carbon of tyrosine becomes the nitrile carbon of the glycoside while the $\beta$-carbon of the amino acid becomes the aglycone carbon that bears the glucosyl group (see Conn and Butler 1969 for review). The nitrogen atom of the aglycone is

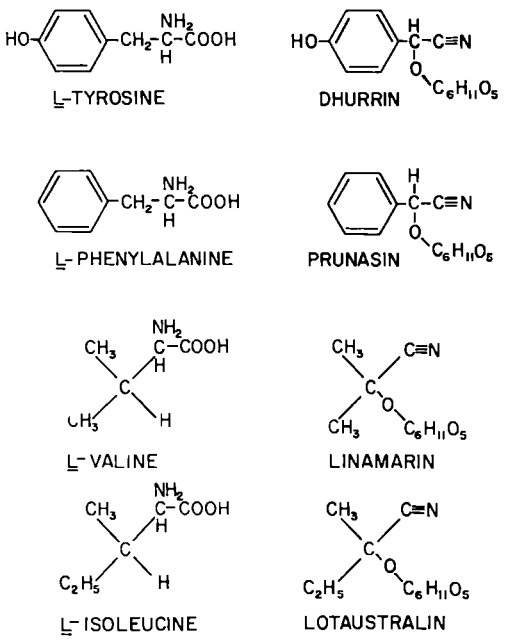


FIG. 2. The precursor–product relationship between certain amino acids and certain cyanogenic glycosides.

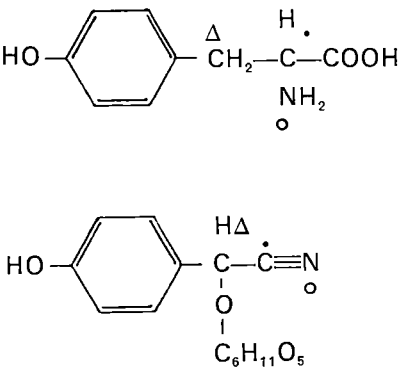

FIG. 3. The origin of certain atoms of dhurrin.

derived from the amino nitrogen of the amino acid. In the case of dhurrin (Uribe and Conn 1966), linamarin (Butler and Conn 1964), and taxiphyllin (Bleichert et al. 1966), double-labelled experiments involving the use of amino acids labelled with $^{14}C$ in the α-carbon and $^{15}N$ in the amino group have shown that, as the amino acid is converted to the glucoside, there is little change in the ratio of specific activities of the two isotopes. This has been taken as evidence that the bond between those two atoms is not severed during the conversion and therefore that all intermediates in the biosynthetic pathway must be nitrogenous in nature. Further, in the case of dhurrin, tyrosine double-labelled in the α- and β-carbon atoms was administered and the ratio of specific activities of the corresponding atoms in the aglycone determined (Koukol et al. 1962). As there was no significant difference in the isotope ratio for those two atoms, it was concluded that the covalent bond linking the α–β carbons was not severed during the biosynthesis.

These observations required a biosynthetic pathway in which the carboxyl carbon of the precursor amino acid is lost while the α-carbon is oxidized to the level of a nitrile (a 4-electron oxidation) and the nitrogen atom is retained. In addition, the β-carbon undergoes a 2-electron oxidation (a hydroxylation, Zilg et al. 1972) to form the hydroxyl group to which the sugar is attached. These requirements and the known nonenzymic conversion of 2-oximino acids into nitriles by a concerted dehydration and decarboxylation led to the testing of $^{14}C$-labelled oximes and nitriles as possible precursors of the cyanogenic glycosides in flax and sorghum (Conn and Butler 1969). The data in Table 2 were some of the earliest to show that the incorporation of isotope from labelled aldoxime, nitrile, and α-hydroxynitrile was remarkably efficient when compared with the precursor amino acid. To account for these observations the biosynthetic pathway (Fig. 4) involving these compounds as intermediates was postulated (Hahlbrock et al. 1968). It should perhaps be pointed out that the sequence of compounds in the pathway was not determined by the relative efficiency with which these compounds were incorporated since they all were about equally effective

TABLE 2. Conversion of oximes and nitriles to linamarin in linen flax. (Data from Tapper et al. 1967 and Hahlbrock et al. 1968.)

| Compound administered | % converted to linamarin |
|---|---|
| *Experiment 1* | |
| L-[U-$^{14}C$]-valine | 25 |
| [U-$^{14}C$]-isobutyraldoxime | 21 |
| [U-$^{14}C$]-isobutyraldehyde | 0.7 |
| *Experiment 2* | |
| L-[U-$^{14}C$]-valine | 23 |
| [1-$^{14}C$]-isobutyronitrile | 11 |
| 2-hydroxy-[1-$^{14}C$]-isobutyronitrile | 28 |

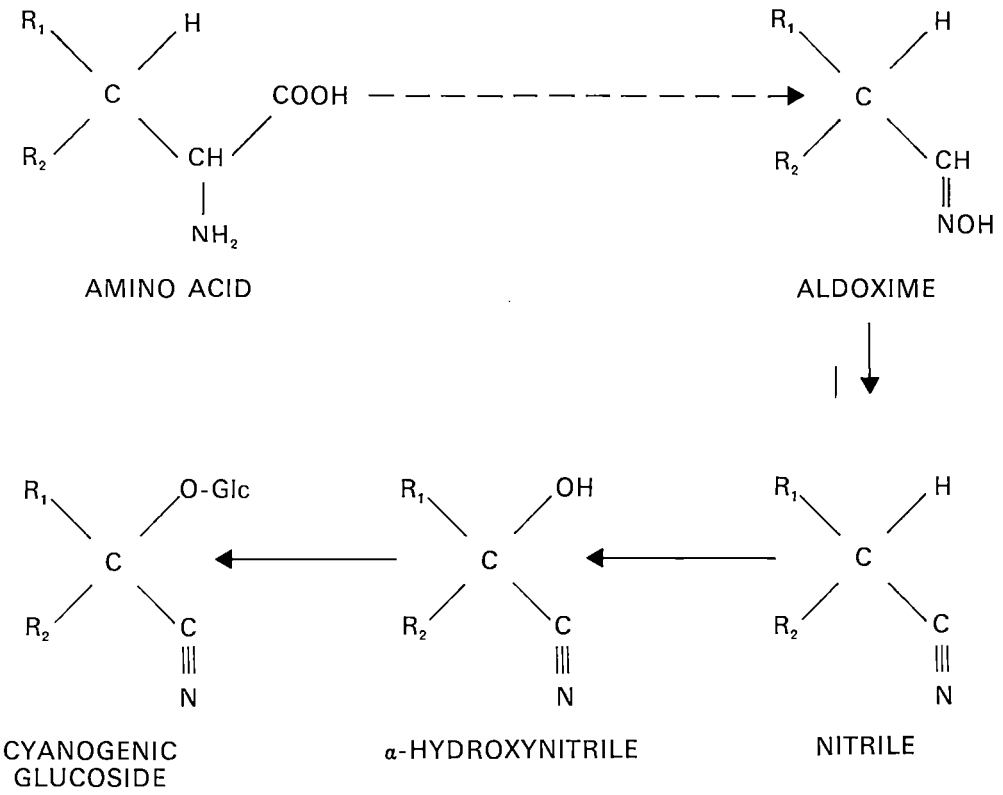


FIG. 4. A possible pathway for biosynthesis of a cyanogenic glycoside from its precursor amino acid. For linamarin, $R_1 = R_2 = CH_3-$, and the amino acid is valine. For lotaustralin, $R_1 = C_2H_5-$, $R_2 = CH_3-$, and the amino acid is isoleucine. For prunasin, $R_1$ = phenyl, $R_2 = H-$, and the amino acid is phenylalanine. For dhurrin, $R_1$ = *p*-hydroxyphenyl, $R_2 = H$, and the amino acid is tyrosine.

when compared with the precursor amino acid. Instead, the sequence was based primarily on the known fact that oximes are readily dehydrated to nitriles and that the cyanohydrin could be glucosylated enzymically.

One might reasonably expect that the postulated intermediates shown in Fig. 4 should be detectable in extracts of untreated cyanophoric plants. This, however, with one exception, has not been possible. The failure to detect these compounds obviously might be due to the analytical methods employed not being sufficiently sensitive to detect very small amounts. An alternative possibility, of course, is that the intermediates remain bound on the surface of the enzyme systems(s) that carry out the biosynthesis.

There is, however, other evidence which supports the existence of the pathway shown in Fig. 4. The first consists of evidence of the sort presented in Table 2 showing that the appropriate aldoximes, nitriles, and α-hydroxynitriles when labelled with $^{14}C$ and administered to cyanophoric plants are effectively incorporated into the cyanogenic glucoside. Data of this sort have been published (Tapper and Butler 1971) for the formation of linamarin and prunasin in linen flax and cherry laurel, respectively, and for the formation of dhurrin in sorghum (Conn 1973). While such data are clearly indicative of these compounds truly being intermediates, they are subject to the criticism that these compounds may, when fed to the plant, be incorporated due to their closely resembling, but not actually being, true intermediates.

A second type of evidence consists of "trapping" experiments in which the suspected intermediate (aldoxime or nitrile) is administered together with the $^{14}C$-labelled precursor amino acid to the appropriate plant. After a period of metabolism, the intermediate is then reisolated from the plant, purified, and examined for radioactivity. In those

instances where radioactivity is found in the intermediate being examined in the trapping experiment, one can conclude that the intermediate can be formed from the precursor amino acid and therefore participate in the postulated pathway. Such data have been published in the case of linamarin biosynthesis (Tapper and Butler 1972) and are available for dhurrin biosynthesis (Farnden et al. 1972).

To the biochemist, the most satisfactory evidence would be the isolation and characterization of the individual enzymes catalyzing the reactions of the biosynthetic pathway. Progress of this sort is being made since the isolation and characterization of an enzyme that synthesizes linamarin and (R)-lotaustralin from 2-hydroxyisobutyronitrile and 2-hydroxy-2-methylbutyronitrile, respectively, was described (Hahlbrock and Conn 1970). Similarly, the isolation and characterization of an enzyme in sorghum that produces dhurrin from the corresponding α-hydroxynitrile has been achieved (Reay and Conn 1968). Both enzymes utilize uridine diphosphate glucose [UDPG] as the glucosylating agent and the type reaction may be represented as:

α-hydroxynitrile + UDPG → UDP + cyanogenic glucoside.

Interesting stereochemical aspects of these reactions have been studied and reviewed elsewhere (Conn 1973). Work is underway to detect and characterize other reactions of the pathway suggested in Fig. 4 and, in the case of the conversion of *p*-hydroxyphenylacetaldoxime to *p*-hydroxyphenylacetonitrile, a particulate enzyme that catalyzes this reaction in sorghum has been detected (Farnden et al. 1972). Its properties are presently under investigation in this laboratory.

A major area of uncertainty in the pathway (Fig. 4) is the reaction(s) by which the amino acid is converted to the aldoxime. This conversion, which involves a 4-electron oxidative decarboxylation of the precursor amino acid may well be identical to the initial portion of the pathway leading from amino acids to glucosinolate compounds (Underhill and Wetter 1966). In the case of these compounds, Kindl and Underhill (1968) have postulated that the corresponding *N*-hydroxyamino acid is an intermediate and have provided some evidence in support of this suggestion. However, this type of compound has by no means been demonstrated as an intermediate in the case of the cyanogenic glucosides and much additional work is required to establish the role, if any, of the *N*-hydroxyamino acid in the biosynthesis of cyanogenic glycosides.

## Function of Cyanogenic Glycosides

No discussion of cyanogenic glycosides is complete without some comment on their role in the plant. Robinson (1930) reviewed the early suggestions which included the cyanogens being nitrogen reserves and precursors for protein synthesis, excretory waste products, and protective substances. Our present knowledge of protein synthesis clearly eliminates the first of these suggestions. Then, too, the suggestion that the cyanogenic glycosides are inert waste products that must be excreted has frequently been criticized. The now fairly extensive literature on the metabolic activity of these compounds and the assimilation of HCN by plants (Conn and Butler 1969) provides basis for further criticism of this second suggestion.

Because the cyanogenic glycosides are not ubiquitous in nature they must be classified as secondary plant products. Therefore, no primary metabolic or physiological role seems likely, although at one point a role in asparagine biosynthesis appeared promising (Castric et al. 1972). Rather, it seems more plausible that during evolution some plants acquired the biochemical ability to synthesize this fascinating group of compounds, and this ability has been maintained because of the survival or protective value which these substances confer upon the plant. This function has been discussed by Fraenkel (1959) for secondary plant products in general and by Jones (1972) for cyanogenic glycosides in particular. A protective function of this sort does not rule out a role for one of these compounds (vicianin) being the source of HCN that in turn can be used in the biosynthesis of β-cyanoalanine and other lathyrism factors (Tschiersch 1966). As more research is performed on the cyanogenic glycosides, we may learn more about the specific roles that they may play in individual species.

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# Physiological and Genetic Aspects of Cyanogenesis in Cassava and Other Plants

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**Abstract** Available data on the pathways for degradation of cyanoglucosides and subsequent fate of the breakdown products in cassava (*Manihot* spp.) and other plants are discussed. Also considered is the degradation of cyanoglucosides after ingestion by animals and parasitic organisms. The physiological and genetic factors which give rise to variations in cyanoglucoside content in plants are also discussed.

**Résumé** Nous examinons de façon critique nos connaissances sur les voies métaboliques de dégradation des cyanoglucosides et le sort subséquent des produits qui en résultent chez le manioc (*Manihot* spp.) et autres plantes. Nous considérons également la dégradation des cyanoglucosides après ingestion par les animaux et par les organismes parasites. Enfin, nous examinons les facteurs physiologiques et génétiques responsables des variations de la teneur en cyanoglucosides des plantes.

## Degradation of Cyanoglucosides

WHEN plant tissues containing cyanoglucosides are crushed or autolysed, an enzymic hydrolysis takes place releasing the sugar moiety and the aglycone. The crushing of the plants probably allows the glucosidase and glucoside to diffuse together and react. The enzymes hydrolysing these glycosides are $\beta$-glucosidases with differing degrees of specificity for the aglycone portion of the compound.

The enzyme system emulsin, isolated from almond kernels, has been reported by Haisman and Knight (1967) to have at least three separate enzymic activities against cyanogenic glucosides. The first converts the diglucoside amygdalin to the monoglucoside prunasin (amygdalin lyase), which is hydrolysed by the second to give the aglycone and glucose (prunasin lyase). The third activity catalyses the dissociation of the aglycone to hydrogen cyanide (HCN) and benzaldehyde (hydroxynitrile lyase synonymous with oxynitrilase below). Emulsin is specific for $\beta$-glucosides and both $\alpha$- and $\beta$-galactosides. As well as being specific for the sugar moiety, it shows specificity for aromatic cyanogenic glucosides, since it hydrolyses linamarin and lotaustralin very slowly (Butler et al. 1965). Emulsin will also hydrolyse noncyanogenic glucosides such as arbutin and salicin.

The glucosidase, linamarase, isolated from linen flax seed, hydrolyses both aromatic and aliphatic cyanogenic glucosides, but not diglucosides such as amygdalin (Butler et al. 1965). Arbutin and salicin are also hydrolysed at appreciable rates. The linamarase extracted from clover leaves could not be purified by the techniques described for linseed linamarase, because of problems of enzyme

stability, and its substrate specificity is not known. Nartey (1968) showed that a crude preparation from cassava leaves showed strong activity against linamarin and lotaustralin, mild activity against salicin, and weak activity against β-methyl glycoside and amygdalin. Hughes (1968a) studied β-glucosidase production in callus tissue from white clover stems. Evidence was obtained from Michaelis constants that two distinct β-glucosidases were produced: a "low-activity" β-glucosidase with a low Michaelis constant and a "high activity" type with a high Michaelis constant. The latter activity was due to linamarase, since no "high-activity" extracts were obtained from callus tissue from linamarase-negative genotypes. In studies on DEAE-cellulose fractions from clover leaves, further evidence for differences in β-glucosidase specific activity between linamarase-positive and linamarase-negative genotypes was obtained (Hughes 1968b).

Although the aglycones formed after hydrolysis of the glycoside reversibly dissociate to HCN and aldehyde or ketone, the reaction is catalysed in plant tissues by oxynitrilases which are most active at *p*H values where the nonenzymic reaction is slow (Conn 1969). The reaction proceeds to completion at physiological *p*H values, is more rapid at alkaline *p*H values, and is also catalysed by cations and amines. The *p*H optimum of the oxynitrilases from sorghum and from bitter almond kernels lies between 5 and 6 (Bové and Conn 1961), a *p*H at which the nonenzymic dissociation is slow. The oxynitrilase activity would thus be more important in tissue extracts whose *p*H was below neutrality.

The enzymic activities bringing about the hydrolysis of cyanogenic glucosides are usually present in such quantity and are so active that a very rapid breakdown of cyanogenic glucoside results in crushing or damaging the tissue. The rapid breakdown of cyanoglucosides by endogenous enzymes is an important factor in the toxicity of these plants to mammals and to pathogenic or parasitic organisms, and these aspects will now be considered in turn in relation to cassava.

With respect to cassava toxicity to humans, there will clearly be marked differences in the extent of linamarase action on the cyanoglucosides, depending on the amount of tissue damage during preparation of the cassava. Peeling off the rind will cause minimal tissue damage and linamarase action, whereas grating will result in maximal tissue damage and hence maximal HCN liberation.

An important additional aspect to consider is the extent to which HCN will be retained as cyanhydrins by reacting with carbonyl groups in various compounds, especially carbohydrates. Cassava root tissues contain appreciable amounts of hexoses (about 4% of the dry matter; Ketiku and Oyenuga 1970) and it can be assumed that cyanhydrins would be readily formed. Linamarin and lotaustralin are not especially acid-labile, and the statements in the literature on the lability of linamarin (Dunstan and Henry 1903, 1906; Collard and Levi 1959; Wood 1966) can be explained in terms of the lability of such cyanhydrins. Where other than fresh plant tissue is analysed, the probability that cyanhydrins are present as a result of cyanoglucoside degradation should be kept in mind. In this connection, the recommendation to add glucose to cassava products to avoid cassava toxicity can only be partially effective, since the glucose cyanhydrin would be dissociated in the intestine with absorption of the cyanide into the bloodstream. de Bruijn (1971) discussed this and showed that glucose additions to cassava root macerates scarcely reduce HCN output. It seems important to us to establish the extent to which cyanhydrins are formed during preparation of cassava food products and to study ways whereby their production is minimised.

Where cyanoglucosides are ingested by ruminants, β-glucosidases from the rumen microflora will also readily hydrolyse the glycosidic bond liberating HCN (Coop and Blakley 1949). The hydrogen cyanide released in the rumen is rapidly absorbed through the wall of the rumen into the blood stream, flowing to the liver where cyanide is detoxified with the formation of thiocyanate. The rate of detoxication by liver tissue in vitro was fast enough to account for most of the cyanide in the rumen of the animal.

The minimum lethal dose of hydrogen cyanide for sheep is 2.4 mg/kg (Coop and Blakley 1949), in agreement with values found for other animals. The minimum lethal dose of lotaustralin: linamarin is 4.5 mg HCN/kg body weight; the tolerance to a higher dose can be ascribed to the time required for consumption of the feed and to the time required for release of cyanide by linamarase, which may be slower in the rumen because of dilution. Over long periods, sheep were able to detoxify and tolerate 3.9 mg HCN/kg per hour for many

hours. No evidence of adaptation to HCN was noticed.

Where the ingestion of forage is relatively slow, as in the normal grazing situation, sheep could well tolerate 15–20 mg HCN/kg body weight per day (Coop and Blakley, 1949). Since clover makes up about 60% of a pasture and the level of glucoside in the leaves is three times that in the petioles which make up 40% of the plant, a critical level of cyanide in the leaves would be 3500 ppm (dry weight) if the sheep ate 1.5 kg dry weight/day. Corkill (1952) bred a strain of white clover with HCN levels approaching 3500 ppm, but no evidence of toxicity to grazing sheep was observed, nor were cyanogenic strains less palatable than acyanogenic clover strains.

In contrast to white clover, sorghum has often caused death of grazing cattle and is regarded as dangerous to feed when the level in the leaves exceeds 500 ppm (dry weight) (Boyd et al. 1938). However, Rose (1941) recorded that cattle continuously grazing sudan grass containing up to 1330 ppm HCN were unaffected, whereas dairy cattle which ate rapidly when put onto sudan grass were affected by lower levels of cyanide. He also noticed that sheep safely grazed sorghum hybrids which had been poisonous to cattle.

Since the maximum level of cyanide in clover pastures might be as high as 1000 ppm, well above a dangerous level in sorghum, it appears that the latter might be more toxic than clover. This could be explained by a faster hydrolysis of the glucoside in sorghum. Coop and Blakley (1949) noted that the presence of sugars greatly reduced the rate of hydrolysis of lotaustralin, so the quantity of free sugar in the forage may also affect the toxicity by retarding hydrolysis of the cyanoglucoside.

de Bruijn (1971) gives levels of HCN in leaves of 15 cassava clones ranging from 540 to 1090 ppm (fresh weight), corresponding approximately to 2000–4000 ppm (dry weight), i.e. considerably higher than for sorghum and sudan grass and tending to be higher than observed for white clover or lotus.

The extent to which liberation of HCN represents a defence mechanism against insects and parasitic organisms should also be considered. Jones (1972) discussed this question and concluded that "though several animals and plants are able to eat or parasitize cyanogenic plants, this does not detract from the possible basic function of cyanogenesis as a defence mechanism. No defence mechanism is absolute and so we can consider cyanogenesis only in comparative and not in absolute terms as a defensive character. It may not be efficient, but it may well be enough to deter many would-be grazers and parasites."

With respect to the metabolism of HCN in plant tissues, it has been demonstrated in most plants studied that HCN can be incorporated into asparagine, with the nitrile group becoming the amide group of asparagine (see review by Conn and Butler 1969). In two cyanogenic plants, it has been deduced from radioisotope studies that $^{14}C$ label from biosynthesised cyanoglucoside can be transferred to asparagine, presumably by degradation of the cyanoglucosides and reassimilation of the liberated HCN into asparagine (Abrol and Conn 1966 for *Lotus* spp.; Nartey 1969 for cassava). It thus appears that the cyanoglucosides are metabolically active and that they "represent some form of storage carbon and nitrogen which are capable of being utilised by the plant" (Nartey 1969). The hypothesis that this represents a mechanism for recovering and recycling nitrogen is an attractive one which needs further evaluation; the alternative is to regard it as a detoxication mechanism rather than a central metabolic pathway, especially as the capability to form asparagine from HCN also exists in plants which do not contain cyanoglucosides.

An additional mechanism for HCN detoxification in plants was reported recently by Chew and Boey (1972), namely the presence of rhodanese activity in crude extracts of cassava leaves. Rhodanese catalyses the formation of thiocyanate from free cyanide and a sulfur donor in animal tissues and some bacterial species, but it has not previously been demonstrated in plant tissues. Chew and Boey calculated that sufficient rhodanese activity was present in tapioca leaves to assimilate any free cyanide released by cyanoglucoside hydrolysis in the cell. The significance of rhodanese in the detoxication of cyanide in plant tissues in relation to the alternative pathway to asparagine described earlier requires further evaluation.

## Genetical Variation in Cyanoglucoside Content

We have been considerably influenced by the recent view of Jones (1972) in preparing this section. In several cyanogenic species, poly-

morphism is exhibited with respect to cyanoglucoside content and such species are of great interest to geneticists in relation to interactions between environment and genotype. Cyanogenic plants contain both cyanoglucosides and the appropriate $\beta$-glucosidase, but plants which are acyanogenic may differ in their cyanoglucoside and $\beta$-glucosidase composition, as follows:

| | *Gross phenotype* |
|---|---|
| Cyanoglucoside + enzyme | Cyanogenic |
| Cyanoglucoside + no enzyme | Acyanogenic |
| Enzyme but no cyanoglucosides | Acyanogenic |
| Neither cyanoglucoside nor enzyme | Acyanogenic |

Jones (1972) states that this scheme has been shown for *Prunus amygdalus, Sorghum vulgare, Trifolium repens*, and probably for *Sambucus nigra*. He stated: "In *T. repens* (Williams, 1939; Corkill, 1940, 1949; Atwood and Sullivan, 1943) and *L. corniculatus* (Dawson, 1941; Bansal, 1966) the presence of both the cyanogenic glucosides linamarin and lotaustralin is determined by a single dominant allele, while the presence of the appropriate $\beta$-glucosidase is also determined by a single dominant allele, at a locus not genetically linked to the glucoside one. With *L. corniculatus* there is the added complication that the plant behaves as an autotetraploid for these loci (Dawson, 1941; Bansal, 1966)."

The environmental factors influencing the frequency of cyanogenesis in *T. repens* have been identified as temperature by Daday (1954a, b) and differential eating by Jones (1962, 1966, 1970). Daday has concluded that low temperatures favour acyanogenic lines while Jones has placed emphasis on the differential eating of cyanogenic and acyanogenic plants by slugs and snails.

In the case of cassava, the balance between cyanogenic and acyanogenic plants in the agricultural situations in which it is grown is tilted so strongly toward cyanogenic plants that acyanogenicity is not recognised. Perhaps the systems of clonal propagation employed agriculturally are stabilising or perpetuating this situation. It does seem likely, however, that a comprehensive screening of cassava seedlings would reveal genotypes which were acyanogenic. The report that an acyanogenic line existed in Indonesian collections but was lost during World War II lends support to this expectation, as do the wide differences (up to 20-fold) in cyanide content reported between clonal lines by many investigators. It would be necessary for the acyanogenic line selected to be of the phenotypes lacking cyanoglucoside; it could also be advantageous for the line to be positive for linamarase, since a high level of this enzyme would be desirable if the line became contaminated in practice. A screening program of genotypes for low or zero cyanoglucoside followed by clonal propagation of the selected material should be possible.

In considering whether breeding for acyanogenesis is desirable, it seems important to distinguish between the requirements for subsistence cultivation so widespread in the tropics and the prospects for more mechanised cultivation which is developing with the increasing use of cassava as an animal foodstuff. Whereas it may be necessary to retain cyanoglucoside in the outer integument of the root for pest protection in the case of subsistence farming, the use of cassava in more intensive agricultural operations might be accompanied by alternative methods of protection against plant pathogens and parasites (systemic fungicides, etc.).

It might be possible to breed a compromise with a level of cyanoglucoside in the outer integument which is sufficiently high to act as a defence against pathogens and parasites, but with a negligible cyanoglucoside content in the main part of the root. Cyanoglucosides are often fairly rigidly restricted to particular organs, e.g. roots of *T. repens* have very low levels and in the passionfruit *Passiflorum mollissima* the cyanoglucoside is present mainly in the rind and rarely in the seeds or the flesh. In our opinion, however, it would seem preferable to breed for complete acyanogenicity, mainly because of the wide variations in cyanoglucoside content which might arise from physiological causes, even in plants bred for low cyanoglucoside content.

An analogy could be drawn with the vigorous and successful plant-breeding program mounted in Canada in recent years to markedly lower the glucosinolate content of rapeseed, and also to completely remove erucic acid which is a major fatty acid constituent in the cultivated varieties of *Brassica napus* and *B. campestris* previously in use. Many thousands of seedlings and seeds were screened in this program and the program of purposeful plant breeding backed up by first-class biochemistry and chemistry represents a first-

class example of how such a program should be approached and carried through. Dr R. K. Downey, plant breeder for the Canada Department of Agriculture in Saskatoon, heads the plant breeding team responsible (see also Kondra and Stefansson 1970).

## Physiological Variation in Cyanoglucoside Content

The literature on cassava reflects some perplexity amongst investigators who have attempted to rationalise the variations in cyanoglucoside content as influenced by physiological factors. This is not surprising since the cyanoglucoside levels will be determined by a number of physiological factors interacting with each other.

### Variation with Age

de Bruijn's (1971) study showed that cyanoglucoside levels are highest in young cassava leaves and petioles, and decline with age. This is a fairly widespread pattern in cyanogenic species. He found no indications that the glucoside concentrations of the tuberous roots are directly related to plant age and considered that fluctuations in glucoside content during growth are mainly due to changes in ecological conditions.

### Water Stress

Darjanto obtained comparative figures (see Bolhuis 1954) for two cassava clones which were each grown on old laterite soil, on two nearby sites. One site had regularly been used as a paddy field; the other drier location had been used for cultivation of upland rice and citronella grass, and had lower fertility especially with respect to signs of potash deficiency. The cyanoglucoside content in roots from both clones were markedly increased (approximately three-fold) when grown in the drier situation, but the apparent differences in soil fertility complicate interpretation. de Bruijn (1971) grew young plants in bags for 2 months, with water regimes which were two-thirds and one-third optimal and observed an increase in cyanide content per unit dry matter in both roots and leaves with increasing dryness. However, he states that in the field the glucoside content would be increased only after a very long dry period, because plants can adapt to short droughts by abscission of some leaves.

### Mineral Nutrition

In general it would be expected that mineral nutritional regimes which increase the pool of nonprotein-nitrogen within the plants, and in particular the valine and isoleucine pools, could lead to increased cyanoglucoside levels, provided there were no direct inhibitory effects of the particular mineral balance or imbalance on the biosynthesis of the cyanoglucosides. Thus a high nitrogen fertilising regime would tend to elevate cyanoglucosides, as observed by de Bruijn (1971) and others. Similarly macro-element and micro-element deficiencies, which often result in the non-protein-nitrogen pool being large, could well cause elevated cyanoglucoside levels. On the lateritic soils on which cassava is so often grown, the frequency of such mineral imbalances seems likely to be high, so that elevated cyanoglucoside contents from this cause would be expected to be common. Potash deficiency would seem to be a particularly likely cause from the literature we have seen.

### Effect of Shade and Ring-Barking

Shading to 35 and 70% daylight for 8 weeks increased the cyanoglucoside content of leaves and correspondingly decreased cyanoglucosides in roots (de Bruijn, 1971), perhaps by reduction of translocation to the roots either of cyanoglucosides or cyanoglucoside precursors. de Bruijn (1971) showed that ringing of stems also increased cyanoglucoside content in the bark above the incision. It would seem important to clarify the extent to which linamarin and lotaustralin are synthesised in cassava root tissue and the extent, if at all, to which they are translocated from the leaves. It may be that the roots, besides acting as a "physiological sink" for carbohydrate, also behave similarly for cyanoglucosides which have been synthesised in the aerial portions of the plant.

## Conclusions

A consideration of the complexity of the physiological interactions governing the cyanoglucoside content of cassava leads us to the conclusion that agricultural practices to reduce the cyanide levels by physiological manipulations are not practicable,

and that genetic manipulation is the only practicable approach.

## Appendix

### The Goitrogenic Effect of Cyanogenic White Clover

In work carried out at this station in the mid 1950s, rats, guinea pigs, and sheep were fed cyanogenic and noncyanogenic white clover (Flux et al. 1956; Butler et al. 1957). Goitrogenic effects were observed in rats and guinea pigs and with rats it was shown by periodic injections of thiocyanate that the levels of thiocyanate encountered in the clover-feeding experiments exerted a direct depressing effect on thyroid gland activity as evidenced by incorporation of $^{131}I$. Serum thiocyanate levels in the two groups of rats were 6.4 mg/100 ml and 15.6 mg/100 ml for noncyanogenic and cyanogenic clover feeding respectively.

In sheep-feeding experiments lasting 43 days, it was shown that serum thiocyanate levels rose to levels similar to those for guinea pigs and rats, but relatively slight goitrogenic effects were observed, namely a reduction in the total iodine per unit wet weight of thyroid. In subsequent long-term grazing experiments where lambs were born from ewes grazing on pure cyanogenic clover, much larger goitrogenic effects were observed in the lambs (Flux et al. 1963) in that thyroid glands were significantly heavier than those of ewes grazed on ryegrass pastures. The goitrogenic effects were not sufficient to influence the productive performance of grazing animals, however.

Serum thiocyanate levels in dairy cows (both lactating and nonlactating) were low and a goitrogenic effect from cyanogenic white clover on these animals appeared unlikely.

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# Biosynthesis of Cyanogenic Glucosides in Cassava (*Manihot* spp.)

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**Abstract** Cyanogenic materials could not be detected in seeds of sweet cassava (*Manihot* spp.) cultivars, whereas low levels of these materials were found in seeds of bitter cultivars. However, both types of seeds synthesised high levels of cyanogens during germination and growth. Linamarin, 2(β-D-glucopyranosyloxy)isobutyronitrile, accounted for 93%, while lotaustralin, 2(β-D-glucopyranosyloxy)2-methylbutyronitrile, accounted for 7% of the total cyanogenic glucosides in cassava. Seedlings efficiently incorporated L-valine-$^{14}$C (U) and L-isoleucine-$^{14}$C (U) into the aglycone moieties of linamarin and lotaustralin, respectively. Appreciable radioactivity from these amino acids were also incorporated into asparagine.

Linamarase, the β-glucosidase which catalyses the hydrolysis of linamarin and lotaustralin, was identified and isolated in crude form from seedlings and leaves of sweet and bitter cultivars. Thus both cultivars contained the enzymes which catalyse the biosynthesis and degradation of the glucosides. The free amino acid profiles of seeds and seedlings indicated that during germination, the action of proteolytic enzymes on seed storage proteins resulted in the rapid accumulation of valine and isoleucine, from which the glucosides were rapidly synthesised. During the growth of seedlings, the concentration of cyanogenic glucosides increased and then fluctuated, without the release of hydrogen cyanide (HCN). Studies with H$^{14}$CN showed that hydrogen cyanide released intracellularly from the glucosides was rapidly incorporated in asparagine, and subsequently into metabolic pools involved with respiration and protein and carbohydrate synthesis.

Cassava plants assimilated H$^{14}$CN as efficiently as $^{14}CO_2$ in the light. The pathway of H$^{14}$CN assimilation was found to proceed by the reaction of cyanide with serine and cysteine, which resulted in the formation of asparagine. Seedling homogenates showed the presence of equally high activities of β-cyanoalanine synthase and rhodanese, the enzymes which catalyse cyanide detoxification. Both enzyme activities were found to be localised in cassava mitochondria, which showed very low sensitivity toward cyanide during respiration.

Electronmicroscopic studies on cassava seed tissues showed the presence of large amounts of fat and protein bodies in all cells. Organelles were little differentiated. At the onset of active cyanogen synthesis, the cytoplasmic organelles were well developed, especially in the roots.

**Résumé** Nous n'avons pu déceler de matériaux cyanogènes dans les graines de cultures douces de manioc (*Manihot* spp.), alors que nous en avons trouvé de faibles quantités dans les graines de cultures amères. Par ailleurs, les deux types de graines synthétisent de fortes quantités de cyanogènes au cours de la germination et de la croissance. La linamarine, 2(β-D-glucopyranosyloxy) isobutyronitrile, est responsable de 93%, alors que la lotaustraline, 2(β-D-glucopyranosyloxy)2-méthylbutyronitrile, est responsable de 7% des glucosides cyanogènes totaux du manioc. Les jeunes plants incorporent efficacement la L-valine-$^{14}$C (U) et la L-isoleucine-$^{14}$C (U) dans la portion

aglycone de la linamarine et de la lotaustraline, respectivement. Une quantité appréciable de radioactivité provenant de ces acides aminés s'incorpore également à l'asparagine.

La linamarase, la β-glucosidase qui catalyse l'hydrolyse de la linamarine et de la lotaustraline, a été identifiée et isolée dans sa forme brute sur de jeunes plants et des feuilles des cultures douces et amères. Les deux types contiennent donc les enzymes qui catalysent la biosynthèse et la dégradation des glucosides. Les profiles d'acides aminés libres des graines et des jeunes plants indiquent qu'au cours de la germination, l'action des enzymes protéolytiques sur les protéines de réserve des graines produit une accumulation rapide de valine et d'isoleucine, à partir desquelles les glucosides sont rapidement synthétisés. Lors de la croissance des jeunes plants, la concentration des glucosides cyanogènes augmente et ensuite varie, sans qu'il y ait libération d'acide cyanhydrique (HCN). Des études avec $H^{14}CN$ démontrent que l'acide cyanhydrique libéré intracellulairement à partir des glucosides est rapidement incorporé dans l'asparagine et par la suite, dans les pools métaboliques impliqués dans la respiration et la synthèse des protéines et des hydrates de carbone.

Les plants de manioc assimilent $H^{14}CN$ aussi efficacement que $^{14}CO_2$ à la lumière. On a découvert que la voie métabolique d'assimilation de $H^{14}CN$ procède par réaction du cyanure avec la sérine et la cystéine, résultant dans la formation d'asparagine. Des homogénats de jeunes plants indiquent la présence d'activités également élevées de β-cyanoalanine synthase et rhodanèse, deux enzymes qui catalysent la désintoxication du cyanure. On a découvert que l'activité de ces deux enzymes est localisée dans les mitochondries du manioc, qui démontrent une très basse sensitivité au cyanure au cours de la respiration.

L'examen au microscope électronique de tissus de graines de manioc révèle la présence, dans toutes les cellules, de fortes quantités de corps contenant des lipides et des protéines. Les organites sont peu différenciés. Au début de la synthèse active du cyanogène, les organites cytoplasmiques sont bien développés, surtout dans les racines.

CASSAVA, manioc, or tapioca (*Manihot esculenta* Crantz, *M. utilissima* Pohl) is one of the most extensively cultivated food plants in the developing countries of the tropics, where its starchy root tubers form a major source of industrial and dietary carbohydrates (and often proteins). The utilisation of cassava root tuber and products as a major staple food presents a variety of health problems. Because of the low protein content, cassava food products have been implicated in the high incidence of Kwashiorkor, the common protein-deficiency syndrome of the developing countries (Jones 1959). The high moisture content and the high ratio of carbohydrates to nitrogen make cassava tubers an excellent substrate for microbial growth and production of high levels of toxic metabolites. It has been shown that *Aspergillus flavus* thrives on cassava meal substrate and produces relatively high levels of aflatoxins (Nartey 1966). These heat-stable carcinogenic metabolites inhibit protein synthesis, and cause liver damage and hepatoma in animals (Butler and Barnes 1963). Thus food products derived from field-dried tubers may represent another health hazard involving protein metabolism in man in the tropics.

A common biochemical feature of the cassava plant is that it synthesises and accumulates cyanogenic materials in its vegetative tissues, especially the edible leaves and tubers. These materials, on hydrolysis, give rise to moderate to lethal concentrations of prussic acid or hydrogen cyanide (HCN), a powerful specific and nonspecific inhibitor of several essential enzyme-catalysed processes, notably the cytochrome oxidase system in respiration (Dixon and Webb 1965). The consumption of cassava products containing high levels of cyanogenic glucosides therefore constitutes a serious health hazard since cyanide released from these food products will act as enzyme poisons. Indeed, cyanide poisoning and death have resulted from the consumption of poorly prepared diets of cassava tubers and products containing lethal amounts of cyanogenic glucosides (Sreeramamurthy 1945; Jones 1959). Furthermore, ataxic neuropathy is endemic in developing countries where cassava products form the major staple food; chronic ingestion of cyanide (cyanogenic glucosides) may contribute to the high incidence of this neuropathological syndrome (Osuntokun 1968).

## Cyanogenesis in Cassava

Tissue of all cassava cultivars so far examined contains cyanogenic glucosides, although in varying concentrations. Variations in the HCN con-

TABLE 1. Concentration of cyanogenic glycosides in tissues of "sweet" and "bitter" cultivars of cassava (data from Nartey 1968).

| Cultivar | Tissue | Cyanogenic glucosides (*mg HCN/kg fresh wt tissue*) |
|---|---|---|
| Sweet (3 varieties) | Seeds | 0.00 |
| | Seedlings (10-day-old) | 285.00 |
| | Leaves (mature) | 468.00 |
| | Roots | 126.50 |
| | Tubers | 402.00 |
| Bitter (3 varieties) | Seeds | 7.50 |
| | Seedlings (10-day-old) | 245.00 |
| | Leaves (mature) | 310.00 |
| | Roots | 185.00 |
| | Tubers | 395.00 |

centrations in tubers, as well as the morphological characteristics of the plants, form the basis of a taxonomic differentiation between the bitter (high HCN) and the sweet (low HCN) cultivars (Rogers 1965). This basis for delineation does not appear to offer an adequate means for differentiating cassava cultivars. Since definite metabolite concentrations are not strictly inherited, the variations in the concentrations of cyanogenic glucosides encountered in different cultivars are probably the result of phenotypic differences and factors such as photoperiodism, thermoperiodism, and nutritional status. These factors reflect the capacity of the species to react in a different manner under any given condition. On the basis of present knowledge, it appears that *Manihot* spp. are genetically cyanophoric, with some dominant cyanogenesis-controlling gene being present in successive cultivars and phenotypes. Thus all cassava varieties probably contain all the enzymes which catalyse the biosynthesis of cyanogenic materials and their degradation. Variations in the concentrations of cyanogenic glucosides in cassava tissues and cultivars may therefore determine enzyme activities, substrate concentrations, and availability, as well as rates of transport, storage, and degradation in specific tissues and cultivars.

A comparative study of cyanogenesis in different tissues of sweet and bitter varieties of cassava showed that seeds of the former contained no detectable amounts of cyanogens, whereas seeds of the latter contained low levels of cyanogens (Nartey 1968). Table 1 shows the variations in the concentrations of HCN evolved from different tissues of six cassava cultivars grown from seed to mature plants. Table 1 also shows that during germination in the dark, seeds of all six cultivars synthesised high levels of cyanogenic materials, and thus provided excellent materials for the study of the biosynthesis of cyanogenic glucosides in cassava. In the light, the concentration of cyanogens increased significantly, but decreased sharply after about 2 days, and then fluctuated without the release of HCN into the closed system. These fluctuations indicated that cassava cyanogens were dynamic metabolites, and that the plants contained the enzymes which catalysed the biosynthesis of the glucosides as well as those which catalysed their degradation. An enzyme with the latter activity was isolated in crude form from various cassava tissues (Nartey 1968). The crude enzyme preparation showed strong activity against linamarin and lotaustralin, mild activity against salicin, and weak activity against β-methyl glucoside.

## Cassava Cyanogenic Glucosides

Until recently, only one cyanogenic glucoside, linamarin, was known to occur in cassava tissues. Linamarin, 2(β-D-glucopyranosyloxy)isobutyronitrile, was isolated and characterised by Dunstan et al. (1906) as a glucoside of 2-hydroxy-isobutyronitrile. That is, on enzymic or non-enzymic hydrolysis, linamarin gives rise to glucose, acetone, and HCN. Butler (1965) showed that most plants containing linamarin also contained a higher homologue of this glucoside, methyl-linamarin or lotaustralin, 2(β-D-glucopyranosyloxy)2-methylbutyronitrile, a glucoside of 2-hydroxy-2-methylbutyronitrile. On hydrolysis, lotaustralin gives rise to glucose, methylethyl ketone, and HCN. Both linamarin and lotaustralin were identified in the roots of *Manihot carthaginiensis* and shown to constitute 96 and 4%, respectively, of the total cyanogenic materials present. Nartey (1968) showed that linamarin and lotaustralin constitute the cyanogenic materials of cassava (*Manihot utilissima, M. esculenta*) in the proportions of 93 and 7%, respectively. The occurrence of lotaustralin (methyllinamarin) with linamarin in cassava tissues was later confirmed by Bisset et al. (1969).

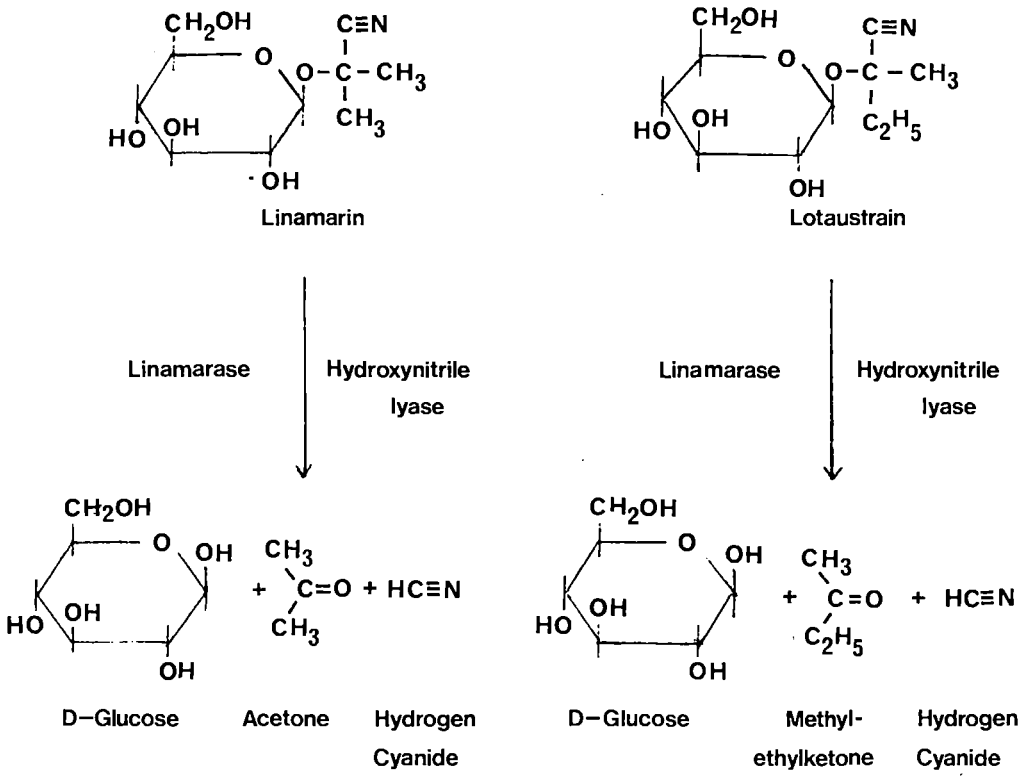


FIG. 1. The structures and hydrolytic products of the cyanogenic glucosides of cassava, linamarin and lotaustralin.

Figure 1 shows the structures and the hydrolytic products of linamarin and lotaustralin.

## Biosynthesis of Linamarin and Lotaustralin

The demonstration in different laboratories that the aglycone moieties of cyanogenic glucosides may be synthesised from structurally related amino acids motivated studies on the effectiveness of valine and isoleucine as precursors of the aglycone moieties of linamarin and lotaustralin, respectively. Results showed that both sweet and bitter cassava seeds contained only trace amounts of valine and isoleucine. However, during germination, large amounts of these amino acids accumulated through the degradative action of proteolytic enzymes on seed storage proteins. Concurrently, the concentrations of linamarin and lotaustralin increased sharply. When uniformly labelled L-valine-$^{14}$C and L-isoleucine-$^{14}$C were administered to cassava seedlings during the period of active cyanogen synthesis (10–14 days), large amounts of radioactivity were subsequently found incorporated in the aglycone moieties of linamarin and lotaustralin, respectively. Table 2 shows the amounts of radioactivity from these amino acids incorporated in the aglycone moieties of cassava glucosides. The table incorporates also radioactivity found in asparagine. The latter is significant with respect to the mechanisms for cyanide detoxification in cassava, which will be dealt with later.

TABLE 2. Incorporation of radioactivity from L-valine-$^{14}$C and L-isoleucine-$^{14}$C into the aglycones of linamarin and lotaustralin, and into asparagine by cassava seedlings (data from Nartey 1969).

| Label administered | Incorporation (%) | | |
|---|---|---|---|
| | Linamarin | Lotaustralin | Asparagine |
| L-Valine $^{14}$C (U) | 13.2 | – | 1.1 |
| L-isoleucine $^{14}$C (U) | – | 2.4 | 0.53 |

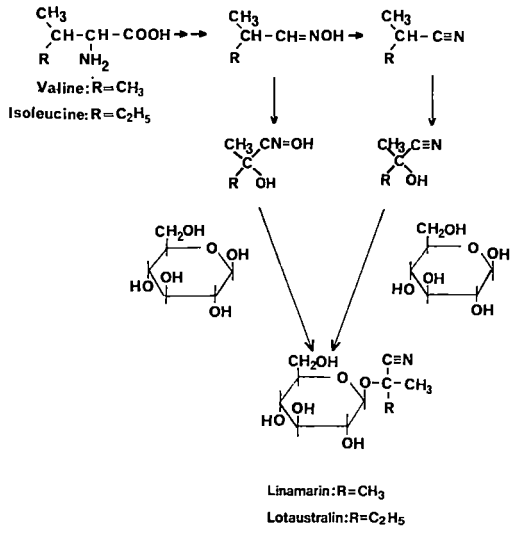


FIG. 2. The biosynthesis of linamarin and lotaustralin in cassava. Pathway incorporates data from Conn and Butler 1969, Nartey 1968 and 1969, and Tapper et al. 1972.

From the foregoing, it is clear that linamarin and lotaustralin are synthesised from valine and isoleucine, respectively, by cassava plants. It is also evident that factors such as the intracellular concentrations of valine and isoleucine, as influenced by protein degradation or synthesis, control the biosynthesis and accumulation of cassava cyanogenic glucosides.

The biosynthesis of linamarin and lotaustralin in cassava proceeds by the pathway illustrated in Fig. 2. Other plant species such as *Trifolium*, *Linum*, and *Lotus* (and *Phaseolus* ?), which accumulate linamarin and lotaustralin during germination and growth, synthesise these cyanogens by the same pathway (Butler and Conn 1964; Abrol and Conn 1966; Tapper et al. 1971).

## Cyanide Detoxification in Cassava

As indicated earlier, the total concentration of cyanogenic glucosides in cassava plants fluctuates with growth period and growth condition. In closed systems, decreases in cyanogen content do not lead to the release of detectable amounts of HCN into the system. Furthermore, cassava plants kept in closed systems containing high levels of HCN appear to grow normally. Under these circumstances, it would appear that cassava plants possess mechanisms for the detoxification of high levels of HCN. The terminal cytochrome oxidase systems of plants, animals, and microorganisms are particularly susceptible to inhibition by cyanide (Dixon and Webb 1965; Bendall and Bonner 1971). Experiments were therefore designed for the investigation of the possible fate of HCN released intracellularly from cyanogens during the growth of cassava plants, and the possible effects of cyanide on the respiration of isolated cassava mitochondria.

## Detoxification and Metabolism of HCN Catalysed by β-Cyanoalanine Synthase

In studies on the fate of HCN in cassava plants, radioactive $H^{14}CN$ was administered to cassava seedlings for various periods of time. In parallel experiments, $^{14}CO_2$ and uniformly labelled acetate-$^{14}C$ were also administered individually to other seedlings. The results of the analysis of extracts from seedlings fed radioactive compounds showed that cassava plants metabolised HCN, $CO_2$, and acetate equally efficiently. However, the patterns of labelling were different. Whereas relatively small fractions of radioactivity from $^{14}CO_2$ and acetate-$^{14}C$ were found in the free amino acid pools, radioactivity from $H^{14}CN$ was predominantly incorporated in the free amino acid pools. A most striking feature of the observed labelling patterns was that although 49% of the total radioactivity from $H^{14}CN$ was located in the free amino acids fraction of seedling extracts, over 95% of the total radioactivity in this fraction was located in asparagine, aspartic acid, glutamine, and glutamic acid. Table 3 shows the amounts of radioactivity from $H^{14}CN$ incorporated into these amino acids by cassava seedlings during various periods of feeding. It is evident from Table 3 that asparagine contained the major fraction of the total radioactivity incorporated into the free amino acid fraction. When labelled asparagine was isolated and degraded, well over 97% of its total radioactivity was located in the amide-carbon atom.

This mechanism for cyanide detoxification operates in a variety of higher plant species (Blumenthal-Goldschmidt et al. 1963). Nartey (1970) also showed that both the carbon and nitrogen atoms of cyanide are specifically incorporated into the amide-carbon and amide-

TABLE 3. Incorporation of radioactivity from $H^{14}CN$ into free amino acids by 10-g cassava seedlings exposed to $H^{14}CN$ released from 2.3 $\mu$mole $Na^{14}CN$, 125 $\mu$ Ci, $8.38 \times 10^6$ cpm, in a closed system for the periods specified (data from Nartey 1969).

| Period of feeding (*min*) | Radioactivity (cpm $\times 10^{-5}$) incorporated in | | | |
|---|---|---|---|---|
| | Asparagine | Aspartic acid | Glutamine | Glutamic acid |
| 10 | 0.86 | 0.11 | 0.16 | 0.07 |
| 60 | 24.91 | 5.73 | 0.89 | 0.18 |
| 180 | 42.03 | 6.68 | 0.65 | 0.43 |

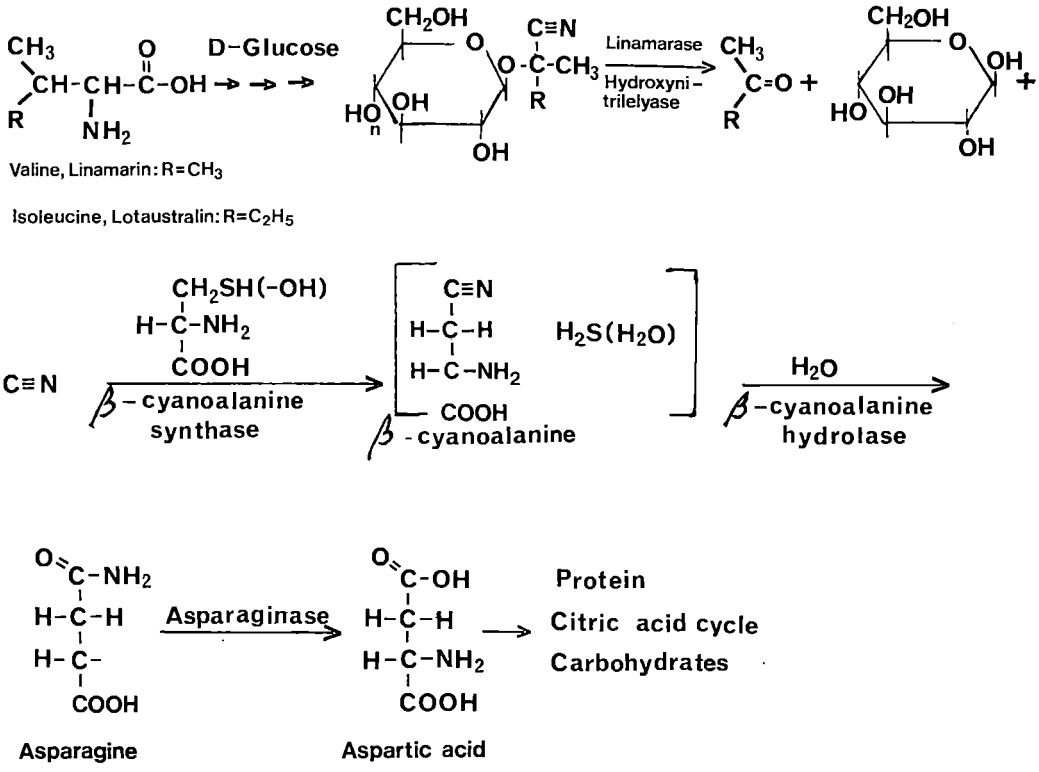


FIG. 3. The detoxification and assimilation of hydrogen cyanide by cassava (Nartey 1969).

nitrogen atoms of asparagine. Both cyanophoric and non-cyanophoric plant species are capable of detoxifying cyanide by this mechanism. Figure 3 illustrates the pathway for the detoxification and assimilation of HCN by cassava plants. In this pathway, both serine and cysteine can act as cyanide acceptors, with the resultant formation of $\beta$-cyanoalanine as the primary reaction product. Since cassava plants contain only trace amounts of cysteine and relatively higher levels of serine, the quantitative data on cyanide incorporation into useful metabolites indicated that serine was the natural cyanide acceptor in cassava. In-vitro experiments with seedling extracts confirmed that serine was a more effective cyanide acceptor than cysteine (Nartey 1969).

Although $\beta$-cyanoalanine is the primary reaction product in this pathway, the compound could not be detected in cassava plants metabolising HCN. Evidently $\beta$-cyanoalanine formed during the assimilation of HCN by cassava was rapidly converted to asparagine, which in turn was readily converted to aspartic acid. High activities of the enzymes which catalyse the hydrolysis of these two

compounds, β-cyanoalanine synthase and asparaginase, have been detected in cassava seedling extracts (Nartey 1972 unpublished data). The operation of these enzyme systems in cassava therefore ensures that the HCN evolved intracellularly from cyanogenic glucosides by the action of linamarase and 2-hydroxynitrile lyase (or by the non-enzyme catalysed dissociation of the hydroxynitrile moieties) is rapidly converted to amino acids, proteins, carbohydrates, lipids, and other cellular materials (Nartey 1969). While this series of enzyme-catalysed reactions provides an excellent means for the conversion of the toxic HCN into non-toxic and useful cellular materials in plants, it probably reflects the capacity of prebiotic systems to cause the non-enzyme catalysed synthesis of amino acids and proteins from the highly reactive HCN and water (Mathews and Moser 1967; Kliss and Mathews 1962).

## Cyanide Detoxification Catalysed by Rhodanese

The most extensively studied mechanisms for cyanide detoxification involve reactions in which cyanide accepts sulfur from inorganic and organic sulfur donors with the resultant formation of thiocyanate. These reactions are catalysed by the enzymes rhodanese (thiosulfate sulfurtransferase) and 3-mercaptopyruvate sulfurtransferase, which occur in plants, animals, and microorganisms (Gemeinhardt 1938; Chew and Boey 1972; Sorbo 1953; Fiedler and Wood 1956; Tabita et al. 1969). Figure 4 illustrates thiocyanate formation from cyanide and inorganic and organic sulfur compounds.

Chew and Boey (1972) reported the occurrence of a rhodanese in cassava leaves. Studies with seedling homogenates and mitochondria have confirmed the occurrence of rhodanese activity in cassava, and indicated that both β-cyanoalanine synthase and rhodanese activities were localised in the mitochondrial fractions of tissue homogenates. β-cyanoalanine synthase has been isolated from the mitochondrial fractions of blue lupine (Hendrickson and Conn 1971). Thus, cyanide detoxification in plants proceeds via the β-cyanoalanine and thiocyanate pathways, depending on the presence of the substrate which acts as cyanide acceptor (serine and cysteine) or sulfur donor (thiosulfate, thiosulfonates and 3-mercaptopyruvate). However, the results of the studies with cassava tissue homogenates and isolated mitochondria indicated that cassava rhodanese activity was inhibited by cysteine, while the β-cyanoalanine synthase activity was equally inhibited by thiosulfate (Nartey 1972). Apparently, only one cyanide detoxification mechanism may operate at a time, since the substrate of the one system inhibits the activity of the enzyme which catalyses the other system.

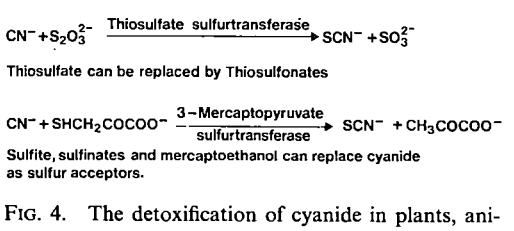


FIG. 4. The detoxification of cyanide in plants, animals, and microorganisms by the formation of thiocyanate.

## Cyanogenesis and Ultrastructural Changes in Germinating Cassava Seeds

As indicated earlier, active biosynthesis and degradation of cyanogenic glucosides in cassava seeds occurred after 10–14 days germination. As a preliminary to the localisation of cyanogenesis in specific cell organelles, electronmicroscopic studies were conducted on non-cyanophoric seeds and cyanophoric seedlings. The results of these studies showed that all tissues of dry and imbibed cassava seeds were filled with fat and large protein bodies. Cytoplasmic and organelle membranes were poorly defined, which made the recognition of plastids and mitochondria difficult. Figures 5 and 6 are electronmicrographs of thin sections of the endosperm and radicle of non-cyanophoric cassava seeds. Figures 7 and 9 are electronmicrographs of thin sections of the cotyledon and root tissues of 10-day-old etiolated cassava seedlings. These figures show that while the cotyledonary tissues still contained many lipid and protein bodies, the root tissues had mobilised these reserve substances, and contained scattered large lipid bodies. In the root cells, proplastids and mitochondria were well differentiated, as were the endoplasmic reticulum and the Golgi apparatus. Figures 8, 10a, and 10b are electronmicrographs of thin sections of the green cotylendonary leaf and root tissues of 17-day-old seedlings which had received light over the

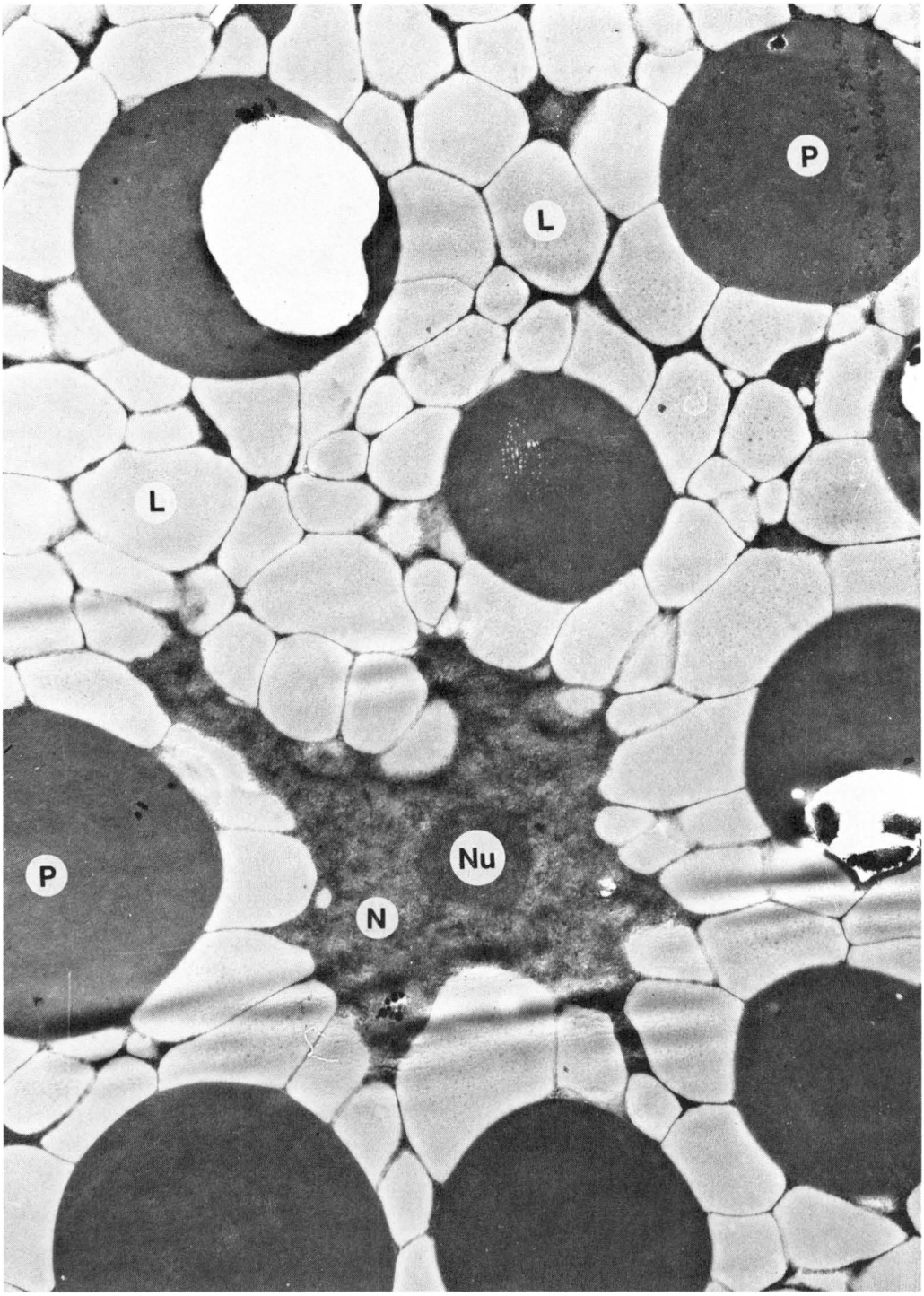


FIG. 5. Electronmicrograph of a thin section through the endosperm of a dry mature cassava seed. The cells are filled lipid bodies (L) and protein bodies (P), Nucleus (N), Nucleolus (Nu). Sections of seed tissues were fixed in 6% glutaraldehyde in phosphate buffer *p*H 7.2, and post-fixed in 2% osmium tetroxide. × 14,000.

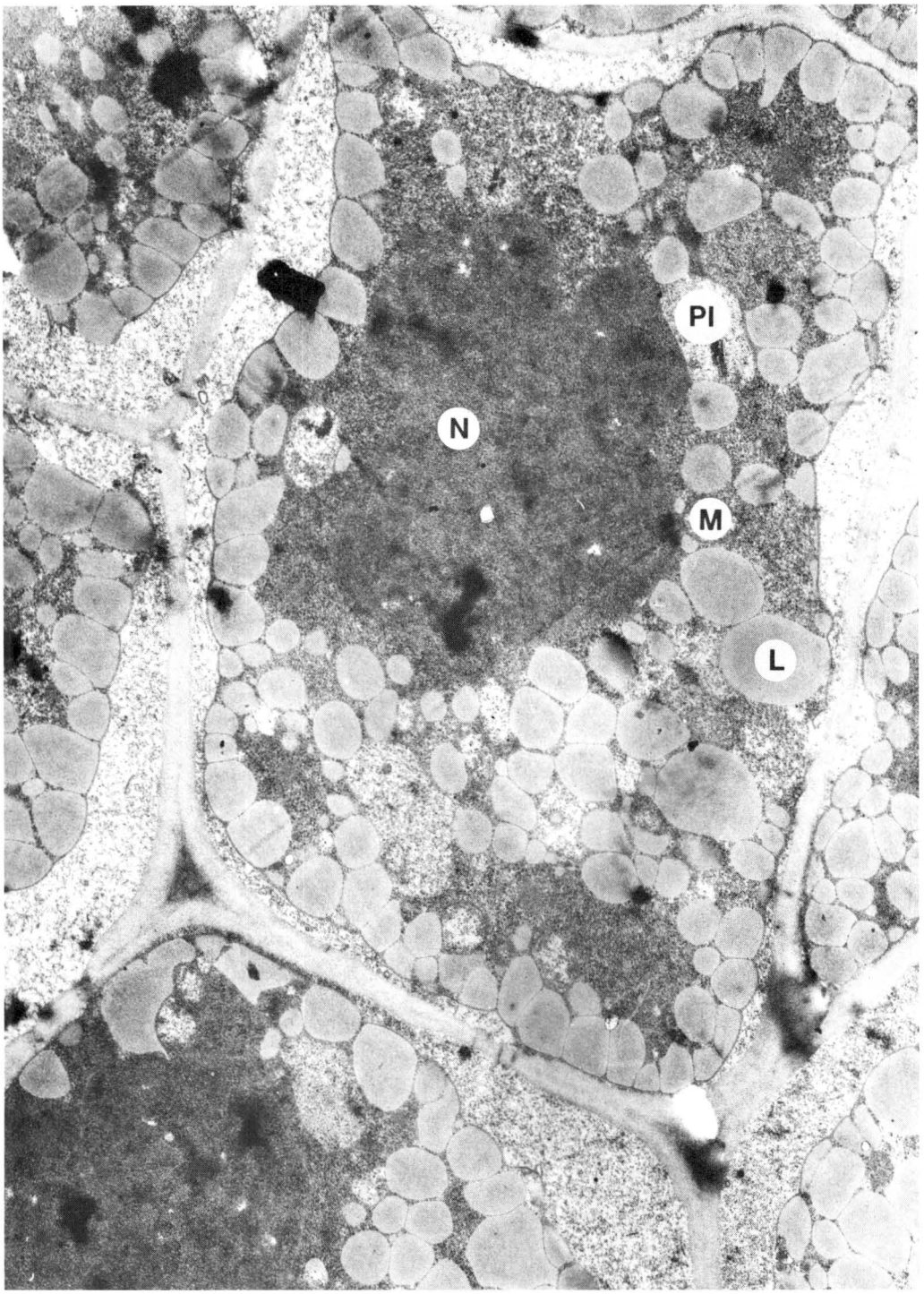


FIG. 6. Electronmicrograph of a thin section through the radicle of a dry mature cassava seed. The cells contain large amounts of lipid bodies (L) in their cytoplasm. Cytoplasmic and organelle membranes are poorly defined. Nucleus (N), Plastid (Pl), Mitochondrion (M). × 14,000.

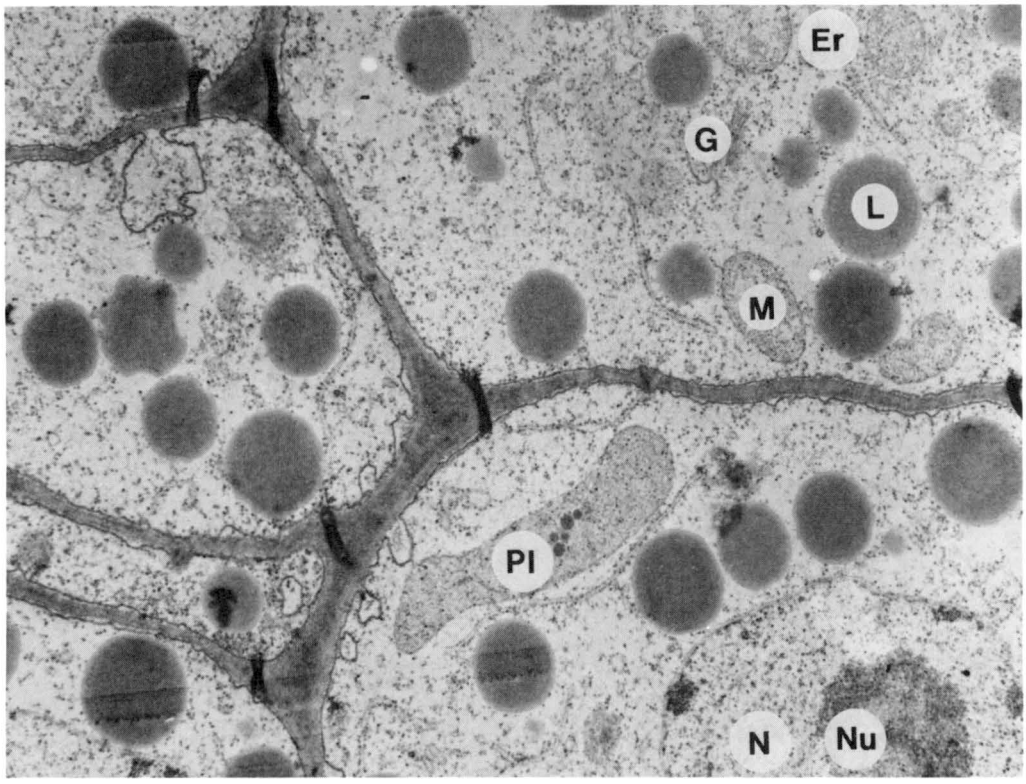


FIG. 7. Electronmicrograph of a thin section through the root of a 10-day-old etiolated cassava seedling. Lipid bodies (L) are scattered through the cytoplasm. Profiles of the endoplasmic reticulum (ER) and Golgi apparatus (G) are well defined. Nucleus (N), Nucleolus (Nu), Plastid (Pl), Mitochondrion (M). × 32,000.

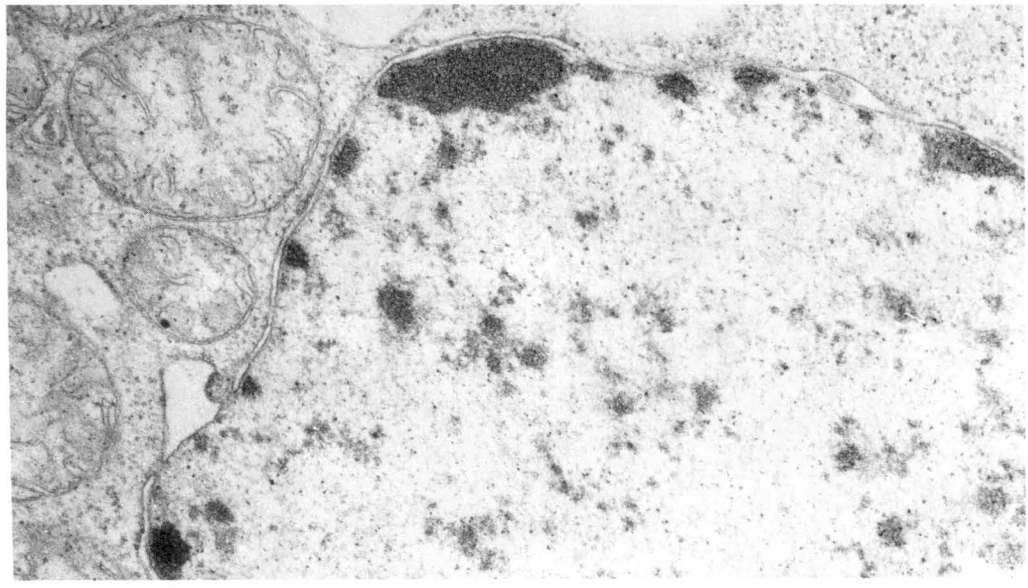

FIG. 8. Electronmicrograph of a thin section through the root of a 17-day-old cassava seedling which had received light over the last 7 days. Cytoplasmic membranes are well defined. Lipid bodies are absent. × 32,000.

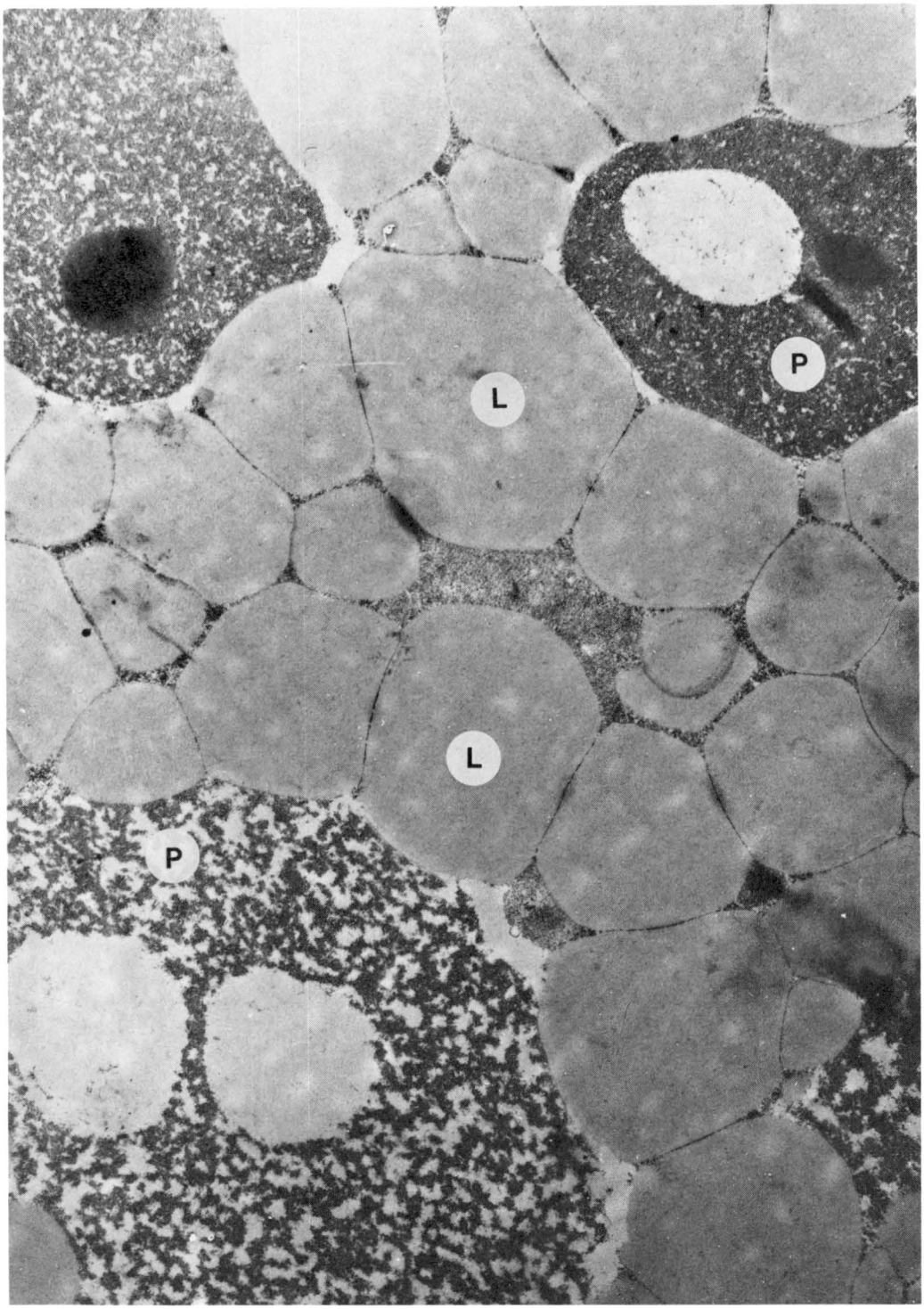


FIG. 9. Electronmicrograph of a thin section through the cotyledon of a 10-day-old etiolated cassava seedling. The cells are packed with lipid (L) and protein (P) bodies. × 14,000.

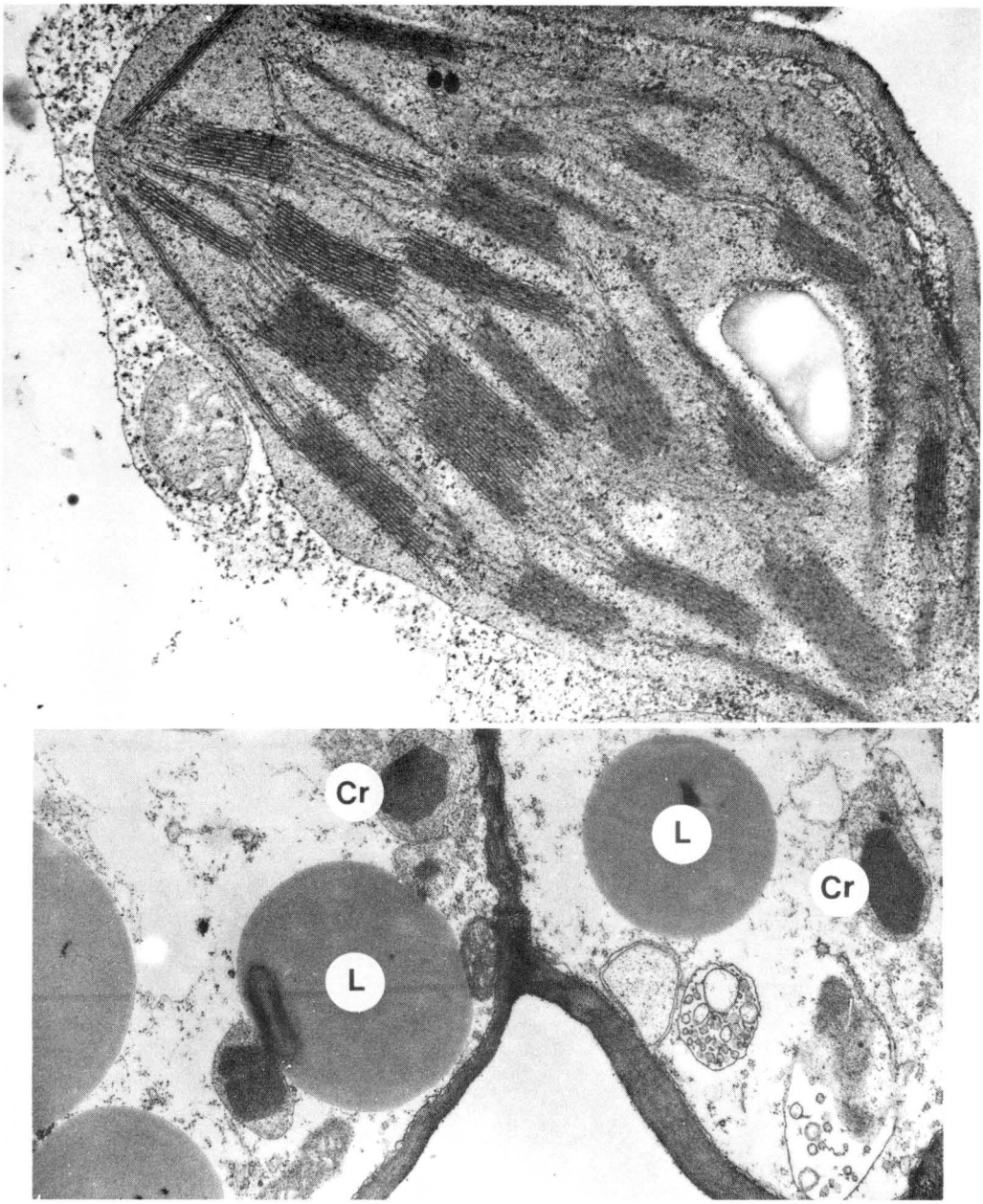


FIG. 10. Electronmicrographs of sections through the green cotyledonary leaf of a 17-day-old cassava seedling which had received light over the last 7 days. The cells contain well-developed chloroplasts (*a*, *top*). Some lipid bodies (L) are still present together with crystalloid containing microbodies (Cr) (*b*, *bottom*). × 28,000.

last 7 days. The root cells showed the absence of lipid bodies and the presence of several well-developed organelles. The leaf cells contained well-developed chloroplasts, some lipid bodies, and crystalloid-containing microbodies.

These analyses reveal that the root cells reach ultrastructural organisation, which is characteristic for an active metabolic state, earlier than the cotyledonary cells. This might also suggest that the biosynthesis and degradation of cyanogenic gluco-

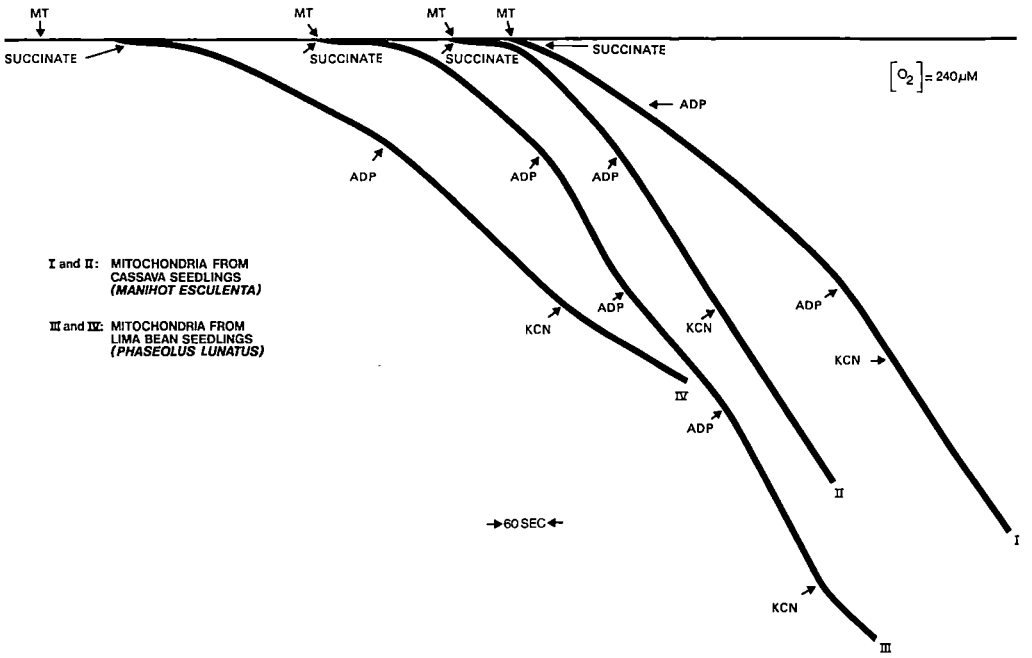


FIG. 11. Typical polarographic traces of the oxidation of succinate by Cassava (A and B) and Lima bean (C and D) mitochondria, showing the effect of neutral KCN on oxygen uptake. Mitochondria were isolated by the methods of Bonner (1967) and Sarkissian and Srivastava (1968). Reaction mixtures contained phosphate buffer *p*H 7.4, 250 μliters mitochondrial suspension (1.2 mg protein), 10 mM succinate, with additions of 225 μM ADP and 0.25 mM KCN. Final volume 3 ml. Temperature 25°C. For ADP:O and RC ratios, see text. A Clark-type oxygen electrode (Yellow Springs Instruments Co.) was used in these traces.

sides in actively germinating cassava seeds occur initially in the roots. Autoradiographic studies are planned to further clarify the organelles involved with cyanogenesis in cassava.

## Effect of Cyanide on Respiration of Isolated Mitochondria

Because the observation that the two enzyme systems (β-cyanoalanine synthase and rhodanese) which catalyse cyanide detoxification in cassava were localised in mitochondrial fractions of seedling homogenates, the effect of cyanide on the respiration of isolated mitochondria was studied. Mitochondria isolated from 14-day-old cassava seedlings and 4-day-old lima bean seedlings oxidised succinate, malate, and NADH. Figure 11 is a recording of oxygen uptake by cassava and lima bean mitochondria, measured with a Clark electrode (Yellow Springs). Lima bean mitochondria were more tightly coupled than cassava mitochondria. ADP:O ratios ranged from 1.5 to 2.0 for cassava mitochondria with succinate as substrate, and from 2.1 to 2.5 for lima bean mitochondria. Respiratory control values ranged from 1.5 to 2.1 for cassava mitochondria, and from 2.0 to 2.6 for lima bean mitochondria. Both types showed a high degree of cyanide-insensitivity during respiration. However, cassava mitochondria were much more insensitive to cyanide than lima bean mitochondria. Oxygen uptake by the former was inhibited by 15.5% in the presence of 0.25 mM KCN (neutralised) while in the latter, the degree of inhibition was 44–50%.

Some plants possess an alternative flavoprotein-mediated cyanide-insensitive respiratory system (Bendall and Bonner 1971). Quite apart from the operation of this system, the activities of β-cyanoalanine synthase and rhodanese in these plants may be involved with the drastic reduction of intracellular cyanide concentrations. Thus, some of the functions of these enzymes may be directly related to the preservation of electron transport via the cytochrome oxidase system, which is highly sensitive to cyanide.

## Conclusions

The available evidence suggests that all cultivars of cassava hitherto studied are cyanophoric, and thus are capable of synthesising and storing lethal concentrations of cyanogenic glucosides in their edible leaves and tubers. The utilisation of these tissues as a source of staple food therefore represents a serious health hazard which can be overcome only by an intensive search for mutant cultivars or varieties in which cyanogenesis is genetically suppressed. An example of varieties of a plant species exhibiting this phenomenon is found in the family Mimosaceae. The South African *Acacia sieberia* var. *woodii* and the Australian *Acacia glaucescence* are both cyanophoric (Rimington 1935; Finnemore and Cox 1928); analysis of organic solvent extracts from leaves and phyllodes indicate that these two plants synthesise and store high levels of the cyanogenic glucosides acacipetalin, sambunugrin, and prunasin. However, the West African *Acacia sieberiana* var. *villosa* does not appear to contain cyanogenic glucosides in its tissues (Nartey 1972 unpublished data).

The production of non-cyanophoric cassava varieties capable of synthesising and storing higher levels of protein in tubers—via mutation studies, intensive screening, and breeding—will undoubtedly minimise the occurrence of diseases engendered by chronic cyanide intoxication and protein defficiency in the developing countries.

## Acknowledgments

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# Assay Methods for Hydrocyanic Acid in Plant Tissues and their Application in Studies of Cyanogenic Glycosides in *Manihot esculenta*

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ZITNAK, A. 1973. Assay methods for hydrocyanic acid in plant tissues and their application in studies of cyanogenic glycosides in *Manihot esculenta*, p. 89–96. *In* Chronic cassava toxicity: proceedings of an interdisciplinary workshop, London, England, 29–30 January 1973. Int. Develop. Res. Centre Monogr. IDRC-010e.

**Abstract** A survey of the methodology of cyanide assay is presented with particular reference to the determination of linamarin, the cyanogenic glucoside of cassava, *Manihot esculenta* Crantz, and some of the problems in obtaining reliable estimates and reproducible data on the potential cyanide yield.

The measurement of potential cyanide output from plant tissues is a convenient method for medical and toxicological studies as it represents an index of health hazard, and therefore, the actual glucoside content receives little attention even in agronomic studies. Linamarin is unusual in that it is not readily hydrolyzed by acid and therefore endogenous or added linamarase must be employed in the release of cyanide. Since the activity of this enzyme in cassava tissues was only recently elucidated, many of the earlier reports on cyanide yield from these tissues are of dubious value.

The peculiarities of the cyanide assay are reviewed in respect to the principal phases of analytical procedure, namely, the release of cyanide from the glucoside, the isolation or recovery of cyanide, and finally, its analytical determination. Because of the reactivity of cyanide ion, its volatility, and the lengthy incubation for enzymic hydrolysis, the crucial point of a reproducible technique is the total release and isolation of cyanide from the substrate and prevention of losses due to the secondary reactions or to the escape of cyanide from analytical train. It is unfortunate that few research papers have concerned themselves with the reproducibility of given methods and their analytical data, and in particular, with the recovery of cyanide added to plant tissue homogenates. The problems also discussed are the errors arising from sampling bulky plant materials, such as cassava roots, and their preparation for analysis, the two aspects of analytical work which in the past have received little attention or are only superficially covered in published reports.

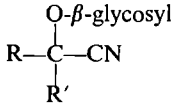
**Résumé** L'auteur fait la revue des diverses méthodes d'analyse du cyanure, en particulier de la linamarine, le glucoside cyanogène du manioc, *Manihot esculenta* Crantz. Il souligne quelques-unes des difficultés à obtenir des estimés fiables et des données reproductibles sur le rendement potentiel en cyanure.

En médecine et en toxicologie, la détermination de la production potentielle de cyanure par les tissus végétaux est une méthode commode, parce qu'elle fournit un indice du hasard à la santé. Ceci explique le peu d'attention portée à la teneur réelle du glucoside, même dans des études agronomiques. La linamarine est exceptionnelle en ceci qu'elle n'est pas aisément hydrolysée par un acide. On doit donc utiliser de la linamarine endogène ou ajoutée pour libérer le cyanure. Comme l'activité de cet enzyme n'a été élucidée que récemment, plusieurs publications antérieures sur le rendement en cyanure de ces tissus ont une valeur douteuse.

L'auteur examine les aspects particuliers du dosage du cyanure en rapport avec les principales étapes du protocole analytique, à savoir, libération du cyanure du glucoside, séparation et récupération du cyanure et, finalement, son dosage analytique. A cause de la réactivité de l'ion cyanure, de sa volatilité et de la longue période d'incubation nécessaire à l'hydrolyse enzymatique, le point crucial de tout processus reproductible est la libération et la séparation totales du cyanure du substrat, et la prévention des pertes dues aux réactions secondaires ou à l'échappement du cyanure dans le train d'appareils analytiques. Il est regrettable que si peu de publications scientifiques se soient préoccupées de la répétabilité des méthodes utilisées et des données analytiques obtenues à l'aide de ces méthodes, en particulier, de la récupération du cyanure ajouté aux homogénats de tissus végétaux. On discute également des problèmes soulevés par l'échantillonnage de matériel végétal volumineux, tel les racines de manioc, et de sa préparation en vue de l'analyse, deux aspects du protocole analytique qui ont reçu peu d'attention ou ne sont que brièvement couverts dans les publications scientifiques.

CYANOGENIC glycosides occur in a great variety of plant species belonging mainly to the Rosaceae and Leguminoseae families, although there is no discernible pattern in their taxonomic distribution throughout the plant kingdom or within an individual species or plant. High levels of cyanogens may be encountered in one particular tissue of a plant while lacking in the same locus in another plant. The presence of the cyanogens in plant foods and forages is of great concern owing to the release of toxic hydrocyanic acid (HCN) through hydrolysis either by acid within the digestive tract or by endogenous enzymes in damaged or disrupted tissues during crop harvest and food preparation.

With a few exceptions, cyanogens in plant tissues are nitriles bound in glycosidic form with aromatic or aliphatic aglycones and possessing a general formula:




Some of the typical cyanogenic glycosides are illustrated in Table 1 (Robinson 1963).

In damaged, disorganized plant tissues, HCN is freed from the glycosides by the action of one or more enzymes which are not necessarily present or active in the same cells or tissues. The sweet almond kernels, for instance, contain the enzymes (emulsin) but not the glycoside. This leaves some hope for the plant breeders in their quest for zero-cyanide or zero-glycoside levels in economic plants as a possible means of eliminating the poisonous attributes of these plants in selected clones or cultivars.

Measurement of cyanide output is of such paramount medical and pharmacological significance that few research papers refer to the actual glucoside content but rather are concerned with the potential yield of cyanide as an index of health hazard.

The primary purpose of this paper is to summarize and evaluate available methodology of cyanide assay in plant tissues, and in particular, to focus on their reliability and some of the problems of sampling and sample preparation. Recent developments in enzymology and instrumentation leave some doubts regarding earlier analytical data on potential cyanide yields. This is particularly true for cassava *Manihot esculenta* Crantz, in which the significance of β-linamarase has been only recently clarified (Butler et al. 1965; Wood 1966).

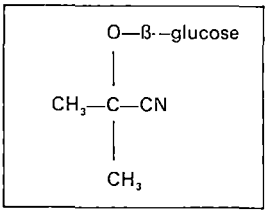
## Methods of Cyanide Analysis in Plant Tissues

Direct assay of cyanogenic glycosides, although not unfeasible, imposes physical limitations and would be too tedious and unreliable to be of any practical value. Because of the medical significance of cyanide, and the relative ease of its detection and determination, it is not surprising that most research data are reported in terms of potential cyanide yields rather than the glycoside content itself. Gas chromatography was used by Bissett et al. (1969) to quantitate linamarin and lotaustralin as trimethylsilyl derivatives, while Butler (1965) estimated the same compounds indirectly on the basis of glucose liberated by β-linamarase. Most assays, however, are based on the determination of HCN liberated by acid or enzyme hydrolysis. One of the inconveniences of this assay is the volatility of HCN (bp 26°C) which necessitates working in a closed analytical train.

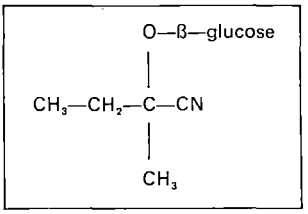
The assays of cyanogens bound with aromatic aglycone components are less difficult to perform

TABLE 1. Common cyanogenic glucosides and their hydrolysis products (McIlroy 1951; Robinson 1963).

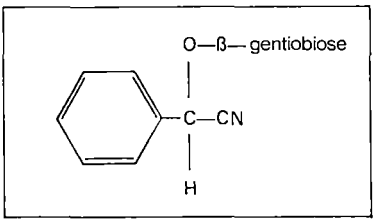
| Glucoside | Sugar | Aglycone |
|---|---|---|
| Amygdalin | Gentiobiose | Mandelonitrile |
| Dhurrin | Glucose | *p*-Oxymandelonitrile |
| Linamarin | Glucose | 2-Hydroxy isobutyronitrile |
| Lotaustralin | Glucose | 2-Hydroxy-2-methyl butyronitrile |
| Prunasin | Glucose | Mandelonitrile |




*LINAMARIN*




*LOTAUSTRALIN*




*AMYGDALIN*

TABLE 2. Cyanide detection limits of various methods.

| Reaction | Source | Detection limit | λ | Procedure |
|---|---|---|---|---|
| Silver nitrate | AOAC 1965 | 0.54–1.08 mg/ml | | titration |
| Picric acid | Snell and Snell 1959 | 5–50 μg | 530 nm | colorimetry |
| Picric acid | Guignard 1906 | 30–50 μg/g | | qualitative |
| *p*-Benzoquinone | Guilbault and Kramer 1965 | 0.6–150 μg/ml | 400, 480 nm | fluorometry |
| Pyridoxal | Takanashi and Tamura 1970 | 0.026–1.3 μg/ml | 356, 432 nm | fluorometry |
| *p*-Chloramine | Pulss 1962 | 0.01–1.0 μg/ml | 582 nm | colorimetry |
| Picric acid | Gilchrist et al. 1967 | 5–50 μg/g | 515 nm | eluate colorimetry |

as they are easily hydrolyzed by acid or exogenous ß-glucosidases which are readily available from commercial sources (assay of dhurrin: Gillingham et al. 1969). The assays of linamarins (Table 1) in flax and cassava are rendered more difficult owing to the apparent requirement of a specific enzyme, ß-linamarase, which is not readily available and is particularly needed for hydrolysis of prepared or processed food materials (Wood 1965, 1966).

Early work on cyanogens stimulated by acute and sometimes lethal toxicity cases (Montgomery 1969) placed fewer demands on cyanide detection limits since it was sufficient to determine potentially toxic cyanide levels. Recent concern about chronic toxicity through continuous intake of low cyanide foods and forages, and the necessity to study the physiological effects of such intake, stimulated the development of procedures which would allow detection and quantitation of trace amounts of cyanide. Some of the detection limits and ranges for various techniques are summarized in Table 2.

There are three distinct steps in the HCN assay: 1) liberation of cyanide through acid or enzymatic hydrolysis; 2) extraction and isolation of the cyanide from the substrate; and, 3) determination of the isolated cyanide.

Since recent research efforts have provided sufficient, though not fully satisfactory, knowledge about cyanogen hydrolysis (Butler et al. 1965; Wood 1966), the isolation and recovery of cyanide from the digest present the greatest difficulty in obtaining reliable and reproducible data. The assay of isolated cyanide presents the least difficulty since most of the available techniques are reasonably well calibrated and tested for reproducibility (Smith 1929; Asmus and Garschagen 1953; Guilbault and Kramer 1965; Gilchrist et al. 1967).

Also, the manner of selecting a representative sample, sample preparation, and handling creates additional problems in obtaining reproducible data. This is particularly true of bulky cassava roots in which concentration gradients exist in

both horizontal and radial directions (de Bruijn 1971).

### Qualitative Tests for Cyanogens

The classical Guignard test (1906) is based on the reaction of alkaline picrate paper with HCN liberated in a closed test tube by spontaneous enzymic hydrolysis of crushed plant material. Cyanide can be detected at 30–50 μg/g concentration. This test has been adapted for quantitation by Boyd et al. (1938), more recently calibrated for colorimetry in the 5–50 μg/extract range by Gilchrist et al. (1967), and proposed for routine cyanogen tests in cassava by Indira and Sinha (1969). Other workable qualitative tests include the ferric ferrocyanide test (Berlin blue) and the benzidine–copper acetate test (Feigl 1960) which according to Wood (1966) detects as little as 0.4 μg of HCN.

### Liberation of Cyanide from Cyanogenic Glucosides

Most of the early methods relied on acid hydrolysis or spontaneous autolysis by enzymes contained in ruptured plant tissues, neither of which appears to give a "total potential yield of cyanide" (Winkler 1951). With acid hydrolysis discrepancies occur due to the formation of amides and ammonia while in spontaneous autolysis the activity of enzymes is no doubt influenced by the conditions prevailing in the heterogeneous plant substrate, the accompanying secondary reactions, and the accumulation of products of hydrolysis. Pulss (1962) attributed the incomplete recovery of cyanide from flax and clover to the secondary binding of cyanide in the digest especially when older necrotic tissues were assayed. Wood (1966) claimed a recovery of at least 87% of cyanide present in the glucoside of cassava when extraneous enzyme was used for hydrolysis. On the other hand, Montgomery (1964) found a 3-h acid hydrolysis at 100°C satisfactory for tropical pulses, and de Bruijn (1971) doubted the necessity of using an enzyme preparation and employed a 24-h spontaneous hydrolysis in cassava macerated to liberate the cyanide.

To minimize these interferences and achieve a near ideal field of potential cyanide, it appears desirable to determine individually for each kind of plant tissue the ideal sample size and duration of autolysis which would not interfere with the action of endogenous or exogenous enzymes to approach a first-order enzyme reaction. This could possibly be tested by a two-step hydrolysis: first an endogenous autolysis followed by a second hydrolysis with added enzyme after the products of first action have been removed by distillation. Ideally, the adequacy of cyanide liberation should be tested by the recovery of added linamarin instead of measuring cyanide recovery added to the substrate as carried out by Pulss (1962).

It is likely that cyanide is quantitatively released from the glucosides of the mandelonitrile type by acid or a combination of acid–enzyme hydrolysis. This does not seem to be true of the linamarins as Wood (1968) claimed that acid does not hydrolyze but produces artifacts, and its only purpose is to expel the HCN from the substrate. In view of the specificity of β-linamarase (Butler et al. 1965; Coop 1940) and the uneven distribution of linamarin in the cassava root (de Bruijn 1971), there is a need for additional study of factors and conditions determining the complete recovery of cyanide from the glucoside. Winkler (1951) pointed out the variability of hydrolytic power of endogenous enzymes and this can be expected in various cassava tissues subjected to glucoside assay especially when different clones are being studied. Hughes (1969) reported differences in β-glycosidase activity in four different clones of white clover. de Bruijn (1971) observed that in low-glucoside clones a 3-h maceration is sufficient whereas in high cyanide cassava clones a 9–12-h maceration is required for autolysis. One wonders whether this may be due to differences in enzyme activity and/or its impairment by products of hydrolysis. Although the conditions and rates of β-linamarase were thoroughly investigated in model systems, without actual testing of plant homogenates, one can only infer and hope that the action is quantitatively similar in the heterogeneous plant substrate undergoing autolysis.

The endogenous enzyme autolysis may be justified in analyzing the outer peel of cassava roots which was used by Wood (1966) to obtain a crude β-linamarase preparation. With cyanide yield from the peeled tuber often below 10–20-μg/g levels, the use of added enzymes seems more than justified since the enzyme activity in that tissue has not been studied.

The duration of autolysis or hydrolysis by added enzyme also suggests a critical point for recovery as there are variations between and

within plant species. In clover, 4–6 h autolysis, and in flax 8–12 h autolysis, were found satisfactory by Pulss (1962); de Bruijn (1971) employed for cassava a 24-h interval for maximum cyanide yields.

Thus the duration of autolysis, the activity of endogenous enzymes in tested plant tissue, and the efficiency of added enzyme merit closer investigation in order to arrive at an ideal method of liberating quantitatively the glucosidic cyanide.

### Extraction and Isolation of Cyanide from Substrate

Cyanide recovery from plant substrate is normally carried out by aspiration with air, nitrogen, or water vapour, and trapping of cyanide in alkaline solution. Critical factors include timing, temperature, volume of substrate, rate of aspiration, sample size, ionic strength, and concentration of solutes in the substrate. The influence of cyanide binding in the substrate has already been mentioned.

According to Wood (1966) the addition of acid merely assists in expelling the cyanide from the substrate. Pulss (1962) found optimum recovery from a mild acid condition (*p*H 5.0) while de Bruijn (1971) and the AOAC method (1965) employ steam distillation without acidification.

Working with flax and white clover, Pulss (1962) carried out a meticulous study on cyanide retrieval from plant substrate including sample size, aspiration, acidity, and interfering substances. Although cyanide is readily and quantitatively recovered from a solution, when added to a clover substrate, as low as 20–30% was recovered with air and 60–80% with nitrogen flushing. A 100% recovery of added cyanide was obtained from clover ash indicating the interference of organic plant substances and oxidative reactions. This clearly underlines the inadequacy of air aspiration and the difficulties in retrieving cyanide from a plant macerate. Reduction of sample size improved the recovery, no doubt due to the dilution of interfering substances. The binding of added nitrogen was particularly pronounced in older, partly necrotic plant material. Recent work at Guelph (Zitnak 1972 unpublished data) indicates a similar binding (10–15%) in acidified cassava substrate with steam distillation. When using a mild acid condition (*p*H 5.0) as recommended by Pulss (1962), these losses are negligible.

With cassava, the most comprehensive study on various conditions influencing analytical data was carried out by de Bruijn (1971). Particular regard was given to sample representation and preservation, time and temperature of autolysis, and sample size for various cassava tissues using steam distillation. In spite of the fact that cyanide recovery was tested with cyanide solution rather than plant macerate, the report provides sound information for future research. It is unfortunate that the cyanide recovery was tested with cyanide solution rather than with plant substrate.

The interferences of organic plant substances occur, according to Pulss (1962), more often with the distillation procedure than with aeration of clover material, however, this is overcome by using the colorimetric method of Asmus and Garschagen (1953) instead of the usual silver nitrate titration. The latter overestimates cyanide yield in clover by 8–14%. Regardless of the accuracy and sensitivity of cyanide determination methods, it is quite clear from the foregoing remarks that the accuracy and reliability of plant assays is primarily dependent on adequate evaluation of the cyanide recovery procedure and its reproducibility, the weight of analytical sample, and the kind of plant tissue to be assayed. It appears quite necessary to perform recovery tests with individual plant species, and even different tissues of the same plant, in relation to their physiological state.

Among recent studies on variability of cyanide yields in cassava, Sinha and Nair (1968) gave no indication concerning sampling, replication, reproducibility of tests, or even the source of assay method that would allow the reader to pinpoint the reliability of the data presented. Similarly, Indira and Sinha (1969) report on a rapid cyanide determination based on the colorimetry of picrate paper reaction (Gilchrist et al. 1967) without an attempt to assess the cyanide recovery from plant substrate. This otherwise simple method appears to have a great potential for routine agronomic studies provided that a method is calibrated with plant substrate tests. There is an obvious need to obtain such data for cassava tissues for this or any other method attempting to determine the potential yield of cyanide as truly representative of the glucoside content. Only in this way can one make a sound comparison of research data from different geographical regions. In cassava, only Wood (1966) attempted to appraise his assay procedure in terms of glucoside content and linamarase

activity. For practical agronomic segregation of high–low cyanide clones, such accuracy may be of lesser concern, however, for the sake of physiological or toxicological studies. It is essential that even in such investigations one should aim for the potential cyanide yield (Winkler 1951) as clearly representative of the glucoside content.

**Determination of Cyanide**

Numerous quantitative methods, differing in sensitivity and detection limits, have been employed in determining the isolated glucosidic cyanide. Since it is beyond the scope of this paper to encompass them in number and in detail, a few general remarks will be made on their applicability in cassava studies. Some indication of sensitivity limits is given in Table 2.

(*a*) Titration with acid or alkaline *silver nitrate* is a well-known standard procedure (AOAC, 1965), suitable for macro determinations (0.5–1.0 mg/ml). Its main disadvantage is in introducing errors through secondary reactions (Pulss 1962) and difficulties in obtaining clear end-points for some plant tissues.

(*b*) Colorimetry with *picric acid* (Snell and Snell 1959): The reaction detects cyanide in the 5–50-$\mu$g range, and although nonspecific due to the interference of other substances, it is applicable to aspiration or distillation extracts of plant tissues. A novel adaptation of this method is the colorimetry of picrate paper eluate as modified and calibrated by Gilchrist et al. (1967). Liberated cyanide reacts with a constant size of saturated alkaline picrate paper and the reaction products are eluted and measured colorimetrically at 515 nm. This test, developed for testing *Sorghum* spp. dhurrin content, was also used by Indira and Sinha (1969) for cassava root and leaf material. Although Indira and Sinha presented only meagre data concerning the variance and reproducibility of measurements, this method can be developed into a useful agronomic routine test. It still needs to be amended to determine cyanide recovery from plant substrate, since Gilchrist et al. (1967) introduced "paper recovery" standards using solely acid-hydrolyzed cyanide solutions for calibration for the assay of dhurrin.

(*c*) Potentiometric measurement with *cyanide-sensing electrode*: A cyanide ion detection electrode with a claim of 15% accuracy (Orion Research Inc. 1967) can detect CN ions in the $10^{-6}$ to $10^{-2}$ M range (0.026–260 $\mu$g/ml). This electrode, which has a multitude of industrial applications, was evaluated for two plant cyanogens, namely in cigarette smoke and in *Sorghum* forage (dhurrin). The method for *Sorghum* (Gillingham et al., 1969) was reported to have a $\pm 0.956$ correlation with picric acid colorimetry of cyanide retrieved by aspiration, unfortunately with an overestimate of 40%. However, Orion Research Inc. have provided a regression line which allows more accurate calculations of the cyanide content. This method merits further evaluation for cassava plant material primarily because of its wide detection range, provided that the endogenous $\beta$-linamarase activity in individual tissues is sufficient for quantitative release of cyanide (the original method employed exogenous $\beta$-glucosidase) and that all cyanide can be retrieved from the plant substrate.

(*d*) Two *fluorometric* methods have been developed to date and both should be applicable for measurement of cyanide in biological fluids since both of them are based on specific cyanide reactions.

Reaction with *pyridoxal* (Takanashi and Tamura 1970) produces a highly fluorescent compound ($\lambda_{ex} = 356$ nm, $\lambda_{em} = 432$ nm) allowing measurements in the $10^{-6}$ to $10^{-5}$ M (0.026–1.3 $\mu$g/ml) range. This appears to be a suitable method for micro-scale study of linamarin distribution in the cassava tuber.

Reaction with *p-benzoquinone* (Guilbault and Kramer 1965) in dimethylsulphoxide ($\lambda_{ex} = 400$ nm, $\lambda_{em} = 480$ nm) has a wide range, 0.6–150 $\mu$g/ml and is suitable for a glass filter fluorometer for routine testing. The disadvantage is the nonaqueous medium, limit of the aqueous sample aliquot to 0.1 ml, and the need to readjust it to a *p*H 7.5 before the reaction. This method is currently being investigated at the University of Guelph with moderate success.

(*e*) A direct cyanide *gas chromatography* is also available but untested on cassava material (Woolmington 1961), although paper and thin-layer and column chromatography were extensively used in the last decade for identification, procedure testing, and plant extract examinations (Bissett et al. 1969; Butler and Conn 1964; Clapp et al. 1966; Wood 1965). Butler (1965) used paper chromatography for indirect assay of linamarin and lotaustralin. These methods require sophisticated laboratory instrumentation and possibilities for routine testing are rather limited. There is, however, a possibility of developing paper or thin-layer chromatography for a densitometric direct assay of the glucosides which is yet to be investigated.

(*f*) Perhaps the *colorimetric* procedure of Asmus and Garschagen (1953) used by Pulss (1962) is worthy of testing on cassava plant material. It is based on cyanide reaction with *p*-chloramine and barbituric acid and is very sensitive, with detection limits of 0.01–1.0 $\mu$g/ml at 582 nm. As mentioned earlier, the method eliminates some interferences normally occurring with steam distillation and silver nitrate titration.

In considering the reviewed methods, the accuracy, detection limits, and reproducibility with cyanide solutions are fairly well established and the main factor in considering their use is the ease of manipulation and technical difficulties. The

matters of most serious concern are, as emphasized earlier, the liberation of cyanide from glucosides contained in the plant substrate and the retrieval of the cyanide after its liberation.

## Problems in Sampling and Sample Preparation

One can not omit from this review a few pertinent remarks on sampling and preparation procedures which in the end are the determinants of reliable data. Too often the sample methodology is treated superficially and much of the accumulated experiences finds little favour and acceptance on the part of editors of research papers unless the methodology is the prime objective of the research. Sample size or representation and replication are too often sacrificed to speed and quick acquisition of data. Papers by Sinha and Nair (1968) and Indira and Sinha (1969) are greatly lacking in this respect as contrasted with de Bruijn's (1971) thorough investigation of analytical conditions and sample materials.

A representative sample for bulky cassava roots is difficult to obtain without homogenization of kilogram quantities of material as done by de Bruijn (1971) who for final analysis used 27-g homogenate samples. In contrast, Wood (1965) used only 4–12-g samples for homogenization and a 1-g sample for the assay proper. There is indeed an urgency to investigate this matter more thoroughly and perhaps the sensible way would be mapping of linamarin distribution within the peeled root in order to arrive at a reproducible method of obtaining a smaller but representative tissue sample. If a uniform sampling procedure could be developed, and approved by common agreement, including as well a standard procedure for liberation and isolation of the cyanide from cassava material, one would have a greater faith in the data reported by various research workers. de Bruijn's (1971) work provides ample directions for sample preparation and preservation.

Perhaps the only other serious concern is whether endogenous $\beta$-linamarase in peeled root tissues is sufficiently active to release quantitatively the cyanide from the glucoside. The use of added $\beta$-linamarase appears imperative for dried or processed cassava products such as gari or konkonte flour (Wood 1966). Since the enzyme is not available commercially the preparation of $\beta$-linamarase according to Coop (1940) might be too cumbersome for obtaining a continuous supply. Present work at Guelph (Zitnak 1972 unpublished data) concerns developing a simplified procedure using 5-day-old flax seedlings to achieve this goal. This procedure is quite successful since greater quantities can be obtained than with Wood's (1966) method using cassava peel as the raw source of the enzyme.

In conclusion, I have attempted to focus attention on some of the problems to be faced by the analyst in the quest for assembling reliable assay data on the occurrence and fluctuation of cyanogens with particular regard to those of *Manihot esculenta*. Hopefully, it will stimulate action and research for the development of standard and uniform analytical procedures.

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# The Mode of Cyanide Detoxication

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OKE, O. L. 1973. The mode of cyanide detoxication, p. 97–104. *In* Chronic cassava toxicity: proceedings of an interdisciplinary workshop, London, England, 29–30 January 1973. Int. Develop. Res. Centre Monogr. IDRC-010e.

**Abstract** The mode of cyanide detoxication in the body is reviewed. The high amount of thiocyanate found in the urine, saliva, and blood of people who eat a lot of cassava was from the detoxication of the cyanide by the enzyme rhodanese which, through combination with thiosulfate or colloidal sulfur, forms a polysulfide chain that can react with cyanide to release sulfur in a suitable form to give thiosulfate. This enzyme represents the chief site of detoxication and occurs in all parts of the body with the largest concentration in the liver.

Cyanocobalamin (vitamin $B_{12}$) occurs in the liver to some extent as the hydroxocobalamin (vitamin $B_{12a}$) which is capable of reacting with cyanide to give cyanocobalamin and hence another important independent pathway for cyanide detoxication. 3-mercaptopyruvic acid, arising from cysteine by transamination or deamination can provide sulfur as rapidly as thiosulfate for cyanide detoxication.

Cystine reacts with cyanide to form cysteine and $\beta$-thiocyanoalanine which tautomerises to 2-aminothidizoline-4-carboxylic acid or its equivalent 2-imino-4-thiazolidine carboxylic acid which is excreted.

Finally the thyroid gland shows some detoxicating effect. In the presence of powdered sheep thyroid the lethal dose of acetonitrite for mice was 1.4 mg/g whereas it was 0.32 mg/g for those not fed the powdered sheep thyroid.

**Résumé** L'auteur passe en revue le processus de désintoxication du cyanure dans l'organisme. La forte quantité de thiocyanate trouvée dans l'urine, la salive et le sang de personnes qui mangent beaucoup du manioc provient de la désintoxication du cyanure par l'enzyme rhodanèse. Cet enzyme, en se combinant avec le thiosulfate ou avec le soufre colloïdal, forme une chaîne polysulfurée qui peut réagir avec le cyanure pour libérer du soufre sous une forme capable de donner du thiosulfate. Cet enzyme est le site principal de désintoxication et se rencontre dans toutes les parties du corps, mais en plus fortes concentrations dans le foie.

On trouve une certaine quantité de cyanocobalamine (vitamine $B_{12}$) dans le foie. Il en est de même de l'hydroxycobalamine (vitamine $B_{12a}$) qui peut réagir avec le cyanure pour donner de la cyanocobalamine et qui est donc une autre voie métabolique indépendante importante pour la désintoxication du cyanure. L'acide 3-mercaptopyruvique, provenant de la cystéine par transmission ou désamination, peut fournir du soufre aussi rapidement que le thiosulfate pour la désintoxication du cyanure.

La cystine réagit avec le cyanure pour former la cystéine et la $\beta$-thiocyanoalanine qui se transforme par tautomérie en acide 2-aminothidizoline-4-carboxylique ou en son équivalent, l'acide 2-imino-4-thiazolidine carboxylique qui est éliminé.

Finalement, la glande thyroïde joue un certain rôle désintoxicant. En présence de thyroïde de mouton en poudre, la dose létale d'acétonitrite chez les souris est de 1.4 mg/g, alors qu'elle est de 0.32 mg/g chez celles qui n'ont pas été nourries de thyroïde de mouton.

A problem that has long been of toxicological and physiological importance is the source of small amounts of thiocyanate found in urine, blood, and saliva. Schmiedeberg (1867) isolated sodium thiosulfate as the barium salt from the normal urine of cats and dogs, and Fromageot and Royer (1945) showed it to be a normal metabolite in higher animals although the mechanism of its formation is obscure. Vassel et al. (1944) found that dogs excreted 2–15 mg thiosulfate–sulfur in 24 h whereas humans excreted 50–125 mg. Gast et al. (1950) reckoned human beings excreted about 20 mg thiosulfate-sulfur in 24 h. At first this was thought to be due to small amounts of this substance present in foodstuffs. Wokes et al. (1952) reported concentrations of 0.1–10 ppm in cow's milk, i.e. about 5.7–57 mg/pint. This source alone can account for a substantial portion of the thiocyanate.

Gemeinhardt (1938) analysed a large number of plants and found that the thiocyanate concentration in all species ranged from about 30 to 950 mg/100 g with the higher figures in cabbage, carrots, and radishes. Wilson (1966) gave much higher figures: 4.1 mg/100 g for sprouts, 1.9 for caneflower, and 0.4 for peas and tomatoes. I found (1970 unpublished data) 0.2–0.5 mg/100 g for cassava products (gari and lafun). The highest values were obtained by Williams (1967 personal communication) for cassava and its products. He obtained 600 mg/100 g for gari, 700–800 for lafun, and 500–600 for yam flour. If we assume these figures are correct, they lend support to the view that thiocyanates are mainly derived from food, increasing with heavy smoking (Lawton et al. 1943) or heavy consumption of beer or strong tea, milk, eggs, and other animal protein sources, all of which contain some preformed thiocyanate (Wokes and Pikard 1955; Wedgewood and Wyatt 1952).

However, if we look at the thiocyanate content of some of the commonly eaten foods in Nigeria, it becomes obvious that ingestion of these will not be sufficient to account for all the thiocyanate observed in ataxic neuropathy. Even if we assume a consumption of 2 kg gari/day, this will only give 8 μmoles thiocyanate/day which will rapidly diffuse through the body fluids and be cleared through the kidneys. On the other hand ingestion of 2 kg gari or lafun will result in about 54 and 200 μmoles of hydrocyanic acid and hence it will be reasonable to assume that the high thiocyanate content is from the cyanide detoxication.

Lang (1894, 1895) showed that injections of cyanide or aliphatic cyanides into rabbit increased the thiocyanate excretion and this has been confirmed by Heymanns and Mesoin (1896). Pascheles (1894) showed by in-vitro studies that when liver or muscle tissue from a dog was digested with NaCN, thiocyanate was produced, the liver tissue being more active than the muscle. Kahn (1912) found from a series of perfusion experiments with liver that the amount of thiocyanate produced increased with the number of perfusion trips and hence confirmed that the liver was an active factor in the production of thiocyanate.

Attention was therefore focused on thiocyanate as a possible detoxication product of cyanide in foods. Lang (1933a, b) postulated the existence of an enzyme he called 'rhodanese' (since it synthesises rhodanate) to be responsible for the reaction under aerobic conditions in the presence of thiosulfate or colloidal sulfur:

$$HCN + Na_2S_2O_3 \ldots\ldots HSCN + Na_2SO_3$$

He found that the enzyme was heat-labile with an optimum *p*H and substrate concentration of 8.3 and 1 mole cyanide:3 moles sodium thiosulfate respectively. The rate of the reaction increases with the temperature up to 38°C and the reaction follows the Schurtz rule, i.e. $K = XN_e t$, where $N_e$ = the concentration of the enzyme, X = the amount of substrate transformed in time $t$, and K is constant. Moreover, the enzyme is widely distributed in all parts of the body but with the liver as the chief site.

Cosby and Summer (1945) purified the enzyme and found that the reaction did not follow the Schurtz rule regardless of the cyanide concentration. Many others have worked on the same line and have found different optimum *p*H values and different distribution patterns in the tissues (Mendel et al. 1946; Bernard et al. 1947a, b; Himwich and Saunders 1948).

The amount of rhodanese in the liver varies with different animals so that detoxication may be expected to take place at different rates. Himwich and Saunders (1948) found the following levels for the livers of animals:

0.78– 1.46 mg/g for dog,
10.08–15.16 mg/g for rhesus monkey,
7.98–18.92 mg/g for rabbit,
14.24–28.38 mg/g for rat.

This probably explains the results obtained by

Mukerji and Smith (1943) that nearly all the cyanide ingested by rabbits was recovered in the form of thiocyanate in the urine in 24–48 h whereas in dogs less than 25% was recovered in 7 days. On the other hand, the activity of the enzyme in parts of the brain and the central nervous system seemed to be the same for the different species of animal and this may account for the LD50 for intravenously injected NaCN to be about the same for all species.

Subsequent work of Saunders and Himwich (1950) has thrown further light on the functions of rhodanese. They proposed that the enzyme forms a loose combination with thiosulfate which breaks down to yield sulfur in a form that can be accepted by the cyanide ion. They explained that the inhibitory effect of certain sulfur-containing compounds like sodium sulfide, dithiobiuret, and cysteine were due to the blocking of the enzyme so that it cannot combine with thiosulfate. They found, in agreement with Lang (1953a, b), that certain divalent cations like $Cu^{2+}$ and $Fe^{2+}$ produce significant inhibitions, whereas others have none. On the contrary, Sorbo (1951a, b) did not obtain any inhibition with cystine alone indicating that the cystine must therefore be easily displaced by thiosulfate giving rise to a situation where thiosulfate, sulfite and other sulfhydryl compounds can react with the active group in the enzyme. This can easily be explained if the active group is a disulfide linkage like the polysulfides whose mechanism of reaction has now been fully worked out, e.g. the first step in the reaction of hexathionate with sulfite or cyanide is ionic displacement of the thiosulfate group by the sulfite or cyanide (Foss 1950):

(a) $^{-}O_3S—S—S—S—S—SO_3^{-} + SO_3^{--} =$

$^{-}O_3S—S—S—S—SO_3^{-} + S_2O_3^{--}$ $\overset{SO_3^{-}}{\cdots\cdots}$

$^{-}O_3S—S—S—SO_3^{-} + S_2O_3^{--}$ etc.

(b) $^{-}O_3S—S—S—S—S—SO_3^{-} + CN^{-} =$

$^{-}O_3S—S—S—S—S—CN + S_2O_3^{--}$ $\overset{*\bar{C}N}{\cdots\cdots}$

$^{-}O_3S—S—S—\overset{*}{S}—\overset{*}{C}N + S\bar{C}N$, etc.

Sorbo (1951a, b, 1953) therefore suggested that rhodanese contains an active disulfide group (rather than –SH groups) which reacts in an analogous way as above by hydrocyanolysis of the first disulfide compound formed by the action of thiosulfate on rhodanese, followed by splitting off of sulfite and formation of thiocyanate:

$$E\begin{matrix}S\\|\\S\end{matrix} + (S\text{–}SO_3)^{2-} \ldots E\begin{matrix}S\text{–}S\text{–}SO_3\\|\\S^-\end{matrix} \overset{CN}{\cdots\cdots} E\begin{matrix}S\\|\\S\end{matrix} +$$

$$SO_3^{2-} + S\bar{C}N$$

The effect of inhibitors can also be explained by this mechanism. Thus when cyanide is first added before thiosulfate or if sulfite and other sulfur-compounds acting as inhibitors are used, this will lead to the formation of a complex which no longer has a disulfide linkage and so is inactive:

$$E\begin{matrix}S\\|\\S\end{matrix} + C\bar{N} \ldots E\begin{matrix}S\text{–}CN\\|\\S^-\end{matrix}$$

From the distribution of the enzyme in the tissues Himwich and Saunders (1948) calculated that the whole liver of a dog can detoxify about 4015 g cyanide and the skeletal muscles 1763 g cyanide to thiocyanate within 15 min, yet only small doses are required for toxicity. They showed that minced tissues converted about twice as much cyanide to thiocyanate as sliced tissues while homogenates converted about 5–17 times as much as sliced and 3–8 times as much as minced tissues and so it appears that permeability factors may play an important part in detoxication irrespective of the quantity of rhodanese. Moreover the reaction does not proceed efficiently unless a thiocyanate concentration of at least three times molar concentration of cyanide is present, a concentration that may not possibly exist in the cell. Thus the availability of sulfur may limit the detoxication possible in vivo. This has been supported by Chen et al. (1934) who found that the injection of thiosulfate is capable of increasing the minimum lethal dose by a factor of 3–4. The high concentration of thiosulfate required is not possible as it penetrates the tissues very slowly whereas cyanide penetrates fast. Giliman et al. (1946) found that 70–80% of injected thiosulfate is excreted unchanged. On the other hand, other sources of sulfur such as cystine, thiourea, sodium sulfide, etc. are not directly effective. Even if it is assumed that certain enzymes convert the sulfur of amino acids to sulfide and then to thiosulfate (Smythe 1942; Fromageot and Monlbasher 1938; der Carabeddian and Fromageot, 1943) this formation will proceed so slowly that it will only be of limited value.

Meister (1953) found that 3-mercaptopyruvic acid might arise from cysteine by transamination or deamination and this compound can provide sulfur as rapidly as thiosulfate for cyanide detoxi-

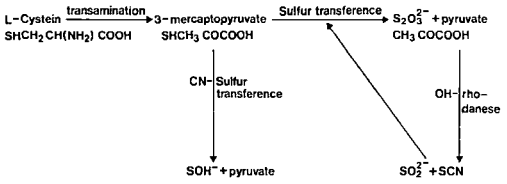


FIG. 1. The cycle formed by the reaction of cyanide with 3-mercaptopyruvate.

cation. He found that crude extracts of liver converted this compound into pyruvic acid and free sulfur at *p*H 7.5–8.5. Since rhodanese utilises only thiosulfate or colloidal sulfur, β-mercaptopyruvic acid could be a sulfur donor. This has been confirmed by Wood and Fiedler (1953) who found that crude acetone extracts of rat liver, incubated at *p*H 9.1, converted cyanide to thiocyanate as rapidly with β-mercaptopyruvic acid as with thiosulfate, requiring the same ratio of reagent:cyanide (3:1) as thiosulfate and the same optimum *p*H of 9.1. Apart from this, cyanide can react with 3-mercaptopyruvate to yield thiocyanate and pyruvic acid (Fiedler and Wood, 1956). The 3-mercaptopyruvate then combines with the cyanide by means of sulfur-transferase to form thiocyanate and pyruvic acid. If sulfite is present, thiosulfate and pyruvic acid are formed from the 3-mercaptopyruvate. The thiosulfate can then be utilised by rhodanese to give sulfite and thiocyanate. The sulfite formed is again ready for use by the sulfur transferase. A cycle is thus formed as shown in Fig. 1.

Although 3-mercaptopyruvic acid has not been detected as a deamination product of cystine, Smythe (1942) found his desulfhydrase to produce pyruvic acid, hydrogen sulfide, free sulfur and ammonia from cystine, a product similar to what had been obtained by Meister (1953) using homogenised rat liver. Administration of $^{35}S$-labelled cystine to animals immediately prior to cyanide dosing yields labelled thiocyanate, confirming the feasibility of this cycle.

In the presence of light, vitamin $B_{12}$ (cyanocobalamin) is converted to vitamin $B_{12a}$ (hydroxocobalamin), the latter can react with cyanide to regenerate vitamin $B_{12}$ (Kaczka et al. 1950):

$$B_{12} \xrightarrow[CN]{\text{light}} B_{12a}$$

The reaction of vitamin $B_{12a}$ with cyanide to form $B_{12}$ is irreversible and so this may be beneficial in cyanide poisoning and thereby provide an independent pathway for cyanide detoxication. The great affinity of vitamin $B_{12a}$ for cyanide is due to the presence of cobalt in the molecule; copper will display the same property. Mushet et al. (1952) injected mice intraperitoneally with KCN preceded or followed by the intravenous administration of vitamin $B_{12a}$ or physiological saline solution. The prophylactic effect of $B_{12a}$ became apparent within 20 sec in reducing or preventing, in some cases, the mortality, respiratory distress, and convulsions. Doses of up to 50–250 mg/kg of $B_{12a}$ were adequate in protecting against 5.5–8.0 mg KCN/kg and if administered not later than 1 min after the KCN dose the above symptoms disappeared immediately and death was prevented. Urine samples collected over a period of 2.5 h showed that about 9.6% of the cyanide administered could be accounted for as vitamin $B_{12}$ and 3.5% as thiocyanate.

Injection of sublethal doses of cyanide to rats causes a significant depletion of the liver store of vitamin $B_{12}$ indicating that this store is an important site of detoxication during cyanide poisoning and hence must be mainly in the form of vitamin $B_{12a}$. The fact that stress conditions such as menstruation, pregnancy, and lactation, which increase the requirement for vitamin $B_{12}$, cause an increase in thiocyanate excretion, confirms the suspicion that $B_{12}$ may be involved directly or indirectly in the formation of thiocyanate in the body. This is further confirmed by the finding that dietary deficiency of vitamin $B_{12}$ leads to increased thiocyanate excretion and that injections of sublethal doses of cyanide to rats cause a significant depletion of the liver store of vitamin $B_{12}$, indicating that this is an important detoxifying agent during cyanide poisoning (Smith 1961).

Vitamin $B_{12}$ contains cobalt in an organic coordination complex, with CN tightly bound to the cobalt. This is shown by the fact that doses up to 1600 mg/kg applied both intraperitoneally and intravenously are nontoxic to mice, despite the fact that this dose is equivalent to 32 mg hydrocyanic acid or eight times the minimum lethal dose for mice (Mushet et al. 1952). These workers therefore proposed vitamin $B_{12}$ as an antidote to cyanide poisoning in mice. This, of course, assumes that some of the vitamin occurs in the hydroxol form which can be replaced by the cyano group. Baxter et al. (1953) showed that ampoules of cyanocobalamin purporting to hold 100 mg of

cyanocobalamin (i.e. vitamin $B_{12}$) contained varying percentages of the hydroxo form. The hydroxo form is known as hydroxocobalamin or vitamin $B_{12a}$. Undoubtedly some vitamin $B_{12}$ exists in the liver as the hydroxo form. Even assuming that all the vitamin $B_{12}$ in the liver occurs in this form, the total amount will be less than 1000 mg (Drouet et al. 1953) and the very small amount of cyanide that can be detoxified will be equivalent to about 25 mg.

It is probable that rhodanese is the main detoxication centre, with its function related to vitamin $B_{12}$ as pointed out by Wokes and Pikard (1955). They proposed that the liver contains some $B_{12}$ in the hydroxo form together with a lot of rhodanese. When cyanide is ingested, both $B_{12}$ and rhodanese compete for it; some is detoxified by rhodanese with the help of sulfur donors, such as sulfur-containing amino acids or their products of metabolism, to thiocyanate which is all excreted in the urine with very little in the faeces (Meister and Pries, 1949). Some of the cyanide combines with the hydroxocobalamin to form cyanocobalamin which then carries out various metabolic functions. Vitamin $B_{12}$ can lose some of the cyanide to supply the 1-carbon fragment for the synthesis of important compounds such as choline and other labile methyl groups and for the conversion of homocysteine to methionine (Kratzer 1953; Stehol et al. 1953; Smith 1954). Some of the cyanide is lost as carbon dioxide in the breath. This has been confirmed by Boxer and Rikards (1952) who demonstrated that labelled $^{14}CN$ given to dogs could quickly be recovered as exhaled carbon dioxide and in the ureide carbon of allantoin as well as in vitamin $B_{12}$ and thiocyanate. Thus it appears that cyanide is incorporated into the 1-carbon metabolic pool probably in the form of formate. The higher activity of formate from the liver than any other constituents makes it a probable intermediate for this conversion with vitamin $B_{12}$ as a possible intermediary, since $^{14}C$ isotope could be recovered from cyanocobalamin.

Finally some cyanide is liberated from vitamin $B_{12}$ by the enzyme cyanocobalamin decyanase which then returns to the liver as the hydroxo form, thus completing the cycle as shown in Fig. 2.

Wokes and Pikard (1955) have also put forward an alternative pathway in which they assumed that excess thiocyanate is present in the tissues compared with cyanide and so it is the thiocyanate that combines with the hydroxocobalamin to form the

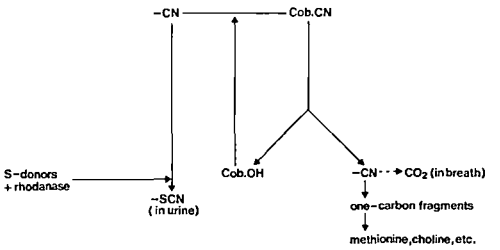


FIG. 2. Hypothetical scheme of cyanide utilization.

thiocyanate derivative, thiocyanate-cobalamin. Although this form has never been isolated from the liver, it may be because it is very labile and less stable than the cyano form and also it would be difficult to distinguish between the two forms. This is as effective as the cyanide form (Buths et al. 1951). Some sulfur is lost from the thiocyanate form so that some $B_{12}$ is left in the cyano form. The sulfur is given up to some active intermediate compound to form a thio compound which may be a precursor of some biologically important sulfur-containing compounds such as sulfur amino acids, glutanthione, or thioacetic acid. The cycle is completed by the loss of CN in vitamin $B_{12}$ which then goes back to the liver in the hydroxo form, and the CN is converted to CNS by rhodanese and is again available for the cycle, as shown in Fig. 3.

This hypothesis is supported by the fact that vegans who are short of vitamin $B_{12}$ detoxify their cyanide to thiocyanate through rhodanese and so they excrete excess thiocyanate. They therefore need more sulfur amino acid donors which could otherwise have been utilised for some other purposes. If methionine is the donor, as has been shown by Hartman (1949) and Hartman and Wagner (1949) on the thiocyanate excretion in liver diseases, it means there will be a decrease in the reserved sulfur in this form. On the other hand if cysteine and cystine are the donors, as shown in sheep by Blakeley and Coop (1949), it will also lead indirectly to methionine deficiency, a sulfur

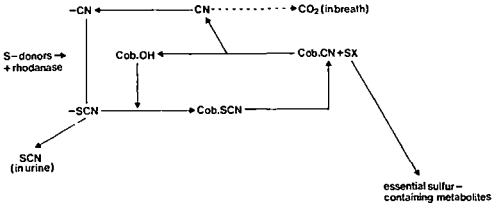


FIG. 3. Hypothetical sulfur transfer cycle.

amino acid in the lens of the eye in which interest has long been centred (Pirie 1956). This is on the assumption that in the presence of $B_{12}$ both cysteine and cystine can act as precursors of methionine as has been shown in *Neurospora* and certain microorganisms by Horowitz (1947), Teas et al. (1948), Fling and Horowitz (1951) and Teas (1950):

$$\underset{\textit{cysteine}}{CH_2(SH)—CH_2CH(NH_2)COOH} + \underset{\textit{homoserine}}{CH_2(OH)CH_2CH(NH_2)COOH} \rightarrow$$

$$\underset{\textit{cystathionine}}{CH_2CH_2CH(NH_2)COOH\ SCH_2CH(NH_2)COOH} \rightarrow \underset{\textit{homocysteine (precursor of methionine)}}{CH_2(SH)CH_2CH(NH_2)COOH} +$$

$$\underset{\textit{serine}}{CH_2(OH)CH(NH_2)COOH} \qquad \underset{\textit{methionine}}{CH_2(CH_3S)CH_2CH(NH_2)COOH}$$

Another pathway of cyanide detoxication was inferred from the observation of Voegthin et al. (1926) that a dose of cystine injected immediately before ingestion of cyanide protected animals from a minimum lethal dose. Later Wood and Cooley (1952) found that cystine alone or in peptide combination converts cyanide to thiocyanate. Thus cystine reacts with cyanide to form α-amino-β thiocyanopropionic acid but this does not release thiocyanate on standing in solution at 37°C. On acetylation or esterification, labilization of the thiocyano group is produced so that on incubation thiocyanate is liberated slowly. The N-acetyl ester is much more labile. Similarly proteins such as egg albumin, plasma, and also glutathione react to produce thiocyanate. This discovery aroused interest in the chemical reaction of cyanide with cystine. Subsequent work showed that cyanide reacts with cystine to split the disulfide linkage to form cysteine and β-thiocyanoalanine (i) which tautomerises to 2-aminothiazoline-4-carboxylic acid (ii) or its equivalent 2-imino-4-thiazolidine carboxylic acid (iii) (Schobert et al. 1951). The reaction takes place spontaneously in vitro (Schobert and Ham 1948):

$$NCS—CH_2—\underset{\displaystyle NH_2}{CH}—COOH \qquad \text{(i)}$$

$$H_2C—\underset{\displaystyle S}{CH}—\underset{\displaystyle \overset{\displaystyle C}{\overset{\displaystyle \|}{N}}—NH_2}{CH}—COOH \qquad \text{(ii)}$$

$$H_2C—\underset{\displaystyle S}{CH}—\underset{\displaystyle \overset{\displaystyle C}{\overset{\displaystyle \|}{NH}}}{CHCOOH} \qquad \text{(iii)}$$

$$\underset{\displaystyle CH—NH_2}{\overset{\displaystyle CH_2SH}{\overset{\displaystyle |}{\overset{\displaystyle |}{COOH}}}} + CNCl — \underset{\displaystyle CH—NH}{\overset{\displaystyle CH_2S}{\overset{\displaystyle |}{\overset{\displaystyle |}{COOH}}}} \diagdown \overset{\displaystyle C=NH}{} \qquad \text{(iv)}$$

A similar compound has been prepared by Aldrich (1951) from the reaction of cysteine and cyanogen chloride (iv). Compound (i) could therefore be a source of thiocyanate in the body since it could undergo oxidative deamination to produce thiocyanopyruvic acid which decomposes readily to yield thiocyanate (Schobert et al. 1951). Further studies by Wood and Cooley (1956) showed that compound (iii) was inert metabolically when administered to rats and hence when cyanide is ingested it combines with the relatively high concentration of free cystine in the blood to form compound (iii) which is then excreted unchanged in the urine and hence provides an independent method of detoxication. This they verified by injecting labelled compound (iii)–$S^{35}$ into rats for 3 days after which they found two radioactive spots in the chromatogram of the urine (which was collected in hydrochloric acid), one corresponding to unchanged compound (iii) and the other to thiocyanate. The latter spot proved to be an artifact caused by the acid. Hence the only possibility of obtaining thiocyanate is when compound (iii) occurs in the open-chain as (i), but the experiment showed the open-chain structures (i) did not occur in equilibrium with the ring structure (iii). The sulfur must, therefore, have been added subsequent to metabolic degradation of cystine to form thiocyanate and not from decomposition of compound (i) as mentioned earlier.

Using cystine–$S^{35}$ Wood and Cooley (1956) found that it produced about 13 times as much compound (iii) as thiocyanate and they recovered about 40% of the labelled sulfur in the form of compound (iii). They also observed this compound (iii) in the saliva of a laboratory worker chronically exposed to a relatively high concentration of cyanide by inhalation. However, the amount of cyanide detoxified is small compared with other pathways. In an experiment without cystine injection, the recovery of intraperitoneally injected

cyanide as thiocyanate and thiazolidine was 80 and 15% respectively.

Another indirect detoxication mechanism that may be mentioned is that of the thyroid gland. Hunt (1905–06) showed that the lethal dose of acetonitrile for mice fed on powdered sheep thyroid was 1.4 mg/g whereas it was 0.32 mg/g for those without thyroid feeding. This indicates that probably thyroid has a detoxicating effect. Later Baumann et al. (1933) found that rabbits injected with acetonitrile excrete 3–5% which was increased on feeding desiccated thyroid. Since thyroidectomy had little effect on the conversion of benzyl cyanide to $SCN^-$, they were of the opinion that the mechanism of detoxication by thyroid depends on the demethylation of acetonitrile.

When hydrocyanic acid is converted to thiocyanic acid there is a 200-fold reduction in toxicity. This may be regarded as a detoxication mechanism in the body which will presumably cope with the small amounts of cyanide formed during metabolism or the minute amounts taken in food, but not with a toxic amount or a large dose introduced artificially into the body.

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# Chronic Cyanide Toxicity in Domestic Animals

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**Abstract** Cases of acute cyanide poisoning are well-authenticated in animals grazing on cyanogenic forages, but chronic toxicity from continuous intake of low levels of cyanogenic plant material, including cassava, has not been clearly identified. Although chronic hydrogen-cyanide toxicity may be influenced by concomitant nutritional deficiencies, such toxicity does not appear as a serious practical problem in the utilization of cassava or cassava products by domestic animals. There is evidence that ataxic neuropathy in humans is associated with high cassava consumption, and that continuous low-level dosage of rats with potassium cyanide will produce lesions in the central nervous system. Such observations have a parallel in the toxic effect of lathrogens and other neurotoxins contained in plants. Some goiters in humans have been attributed to cassava consumption, and experiments with animals strongly suggest that thiocyanate formed during the detoxication of ingested cyanide interferes with the utilization of iodine for thyroxin production. There are interesting similarities between cyanogenic glucosides found in cassava and other plants and the glucosinolates found in *Brassica* species. The latter are, however, the more complex compounds and can yield a greater variety of hydrolytic products.

**Résumé** Les cas d'empoisonnement aigu par le cyanure sont bien prouvés chez les animaux broutant des fourrages qui contiennent du cyanogène, mais la toxicité chronique de matériel végétal à faible teneur en cyanogène, y compris le manioc, ingéré de façon continu, n'a pas encore été clairement identifiée. Quoique la toxicité chronique de l'acide cyanhydrique puisse être affectée par des déficiences alimentaires concomitantes, elle ne semble pas, dans la pratique, causer de problèmes sérieux dans l'utilisation du manioc ou de ses produits par les animaux domestiques. On a des indications que la neuropathie ataxique chez les humains est liée à une forte consommation de manioc. On sait de plus que les rats soumis à des dosages continus d'une faible quantité de cyanure de potassium développent des lésions au système nerveux central. Ces observations ont leur parallèle dans l'effet toxique des lathrogènes et autres neurotoxines contenues dans les plantes. Certaines formes de goitre chez les humains sont attribuables à la consommation de manioc, et des essais sur des animaux suggèrent fortement que le thyocyanate formé au cours du processus de désintoxication du cyanure ingéré entrave l'action de l'iode dans la production de thyroxine. Il existe des ressemblances intéressantes entre les glucosides cyanogènes du manioc et autres plantes et les glucosinolates présents chez les espèces du genre *Brassica*. Ces derniers sont toutefois des composés plus complexes qui donnent, à l'hydrolyse, une plus grande variété de produits.

THERE are no reviews dealing specifically with cyanide toxicity in domestic animals. However, there are several reviews which relate to the basic role of hydrocyanic acid (HCN) in nutrition, particularly those of Montgomery (1965, 1969) and Oke (1969).

Many species of plants contain HCN, usually in the form of cyanogenic glycosides which release

HCN on hydrolysis. At least 300 species of plants tested yield HCN with 52 species in the family Leguminosae and 25 in Gramineae (Quisimbing 1947). The following major species have been associated with HCN poisoning of animals: millet (*Sorghum vulgare*), sudan grass (*Sorghum sudanense*), Johnson grass (*Sorghum halepense*), star grass (*Cynodon plectostrachyum*), arrow grass (*Triglochin maritima*), lucerne (*Medicago sativa*), white clover (*Trifolium repens*), blue couch grass (*Cynodon incompletus*), sedge (*Carex vulpina*), linseed (*Linum usitatissimum*), reed sweet grass (*Glyceria spectabilis*), common vetch (*Vicia sativa*), lima bean (*Phaseolus lunatus*), and cassava (*Manihot esculenta*).

The amount of HCN which can be released from a given species is influenced by many factors. Apart from the fact that some varieties of a species characteristically yield little or no HCN, conditions of drought or wilting, stage of growth, and soil fertility may affect the toxicity (Winks 1940; Anonymous 1934; Lahke 1955; Acharya 1933; Favero 1953; Guisti 1935). Subsequent treatment of the crop may affect the amount of HCN. For example, *Sorghum vulgare* grown under drought conditions may contain dangerous levels of HCN but drying for several days, or ensiling, will reduce the HCN to zero (Kehar and Talapatra 1947). Also, there is evidence for a diurnal variation in the HCN content of plants. Taranenko (1958) and Wolf and Washko (1967) found that HCN concentration reached a peak at midday or early afternoon when photosynthesis was at a maximum.

## Feeding Experiments with Cyanogenic Species

It is necessary to distinguish between chronic and an acute condition of poisoning which is usually fatal.

Acute poisoning from the consumption of cyanogenic plants has been the subject of many reports and is a well-established phenomenon. Many plants, since they serve as forages, or are contaminates of forages, are more of a risk to grazing animals than to poultry and swine which are, to a large degree, fed formulated diets. Surprisingly, there are some known carriers of cyanogenic glycosides which rarely seem to produce acute toxic reactions. A striking example is linseed meal. This protein supplement is fed widely to ruminants and pigs at quite high levels but there are very few reports of toxic reactions in these animals. However, chicks and poults consistently show depressed growth and increased mortality when they are fed linseed meal but these effects can be prevented if the meal is let stand in water at 28–37°C before feeding (MacGregor and McGinnis 1948; Kratzer 1949). It is not known if the toxic factor thus removed or inactivated is HCN arising from linamarin, although HCN may well be involved. Probably during the processing of flax seed to linseed meal there is sufficient heat application to destroy the linamarase thus preventing, in most samples, the subsequent release of significant amounts of HCN.

In contrast to acute poisoning, chronic toxicity, which could be the more serious problem, is largely unresolved. It might be assumed that when fatal toxicity has occurred in certain individuals in a herd or group many others may be affected less obviously. However, controlled feeding experiments are necessary to detect effects which may be exhibited superficially only by somewhat reduced weight gains and feed efficiency.

Feeding trials with cattle and sheep using a variety of plants with cyanogenic potential have, in general, failed to clearly indicate chronic toxicity. Cassava fed in various forms to cattle and sheep, including tapioca meal and chopped-up roots, has not produced obvious ill effects (Brouwer 1932; Anonymous 1937; Assis et al. 1962; Mathur et al. 1969).

Flux et al. (1956) and Butler et al. (1957) found that feeding white clover containing cyanogenic glycosides equivalent to 129 mg of cyanide per 100 g of dry matter to sheep produced no deleterious effect on weight gains, but raised the level of thiocyanate in the plasma, evidence for a metabolic effect from the toxic principle. No strong evidence of a goitrogenic effect was obtained in these studies.

Results with swine are more variable. There are a number of reports in which large amounts of cassava in various forms have been fed to swine with satisfactory results and no evidence of HCN toxicity (Anonymous 1920; Mondonedo and Bayan 1927; Mondonedo 1928; Mondonedo and Alonte 1931; Woodman et al. 1931; Alba 1937). On the other hand, Peixoto (1965) and Velloso et al. (1965–66) found reduced gains as the level of cassava in the swine diet was increased.

Workers at the Centre for Tropical Agriculture, Colombia (Maner and Buitrago 1964; Maner and

Jiménez 1967; Maner et al. 1967, 1970; Maner 1971) have investigated the use of fresh, dried and finely ground and ensiled cassava roots for growing and finishing swine and for gestation and lactation.

In general, cassava was a satisfactory replacement for corn in practical diets. A possible exception was the dried and finely ground cassava which gave a decrease in weight gains proportional to the level of cassava used, with the maximum depression being about 10% when a diet containing 60–70% level of cassava was compared to the control diet. The reason for the growth depression was not clear, but in this experiment, and in others conducted by the Colombian workers, there was no evidence that cassava was contributing a toxic level of HCN to the diets.

There are several publications which indicate that levels of cassava meal over 10–15% in the diet of chicks will reduce gains (Vogt and Penner 1963; Vogt and Stute 1964; Yoshida et al. 1966; Rendon et al. 1969), but adult hens seem to be quite tolerant and maintain egg production even when fed tapioca leaves with a high HCN content (Jalaludin and Yin 1972; Hamid and Jalaludin 1972). The effect of linseed meal in poultry diets and the effect of soaking with water have already been mentioned. The soaking procedure is of considerable interest since Yoshida et al. (1966) found that soaking cassava meal in water overnight reduced its growth-retarding effect on chicks, a result which they attributed to a lessening of HCN toxicity.

It is not possible in most of the publications referred to above to relate the effect of the plant material on weight gains to the intake of cyanogenic glycosides or HCN. HCN was present in the diet in some experiments but in most this information was not obtained or recorded. A number of investigators who recorded good production with potential cyanogenic material reported an absence of gross pathological evidence for HCN. With the exception of the reports by Flux et al. (1956) and Butler et al. (1957) none of the feeding experiments with domestic animals reviewed here evaluated the effect on thyroid tissue or reported metabolic effects which might be related to HCN ingestion.

When plant material is evaluated for its toxic potential, careful attention must be given to the diet composition. It is obvious that inferior gains given by diets high in potentially cyanogenic plant material cannot necessarily be attributed to HCN ingestion. For example, Maust et al. (1969) found that poor gains and parakeratosis in pigs fed a diet containing 36% cassava meal was corrected when extra zinc was added to the diet, even though the level of zinc and its proportion to calcium in the unsupplemented diet were adequate on the basis of accepted standards. Choo and Hutagalung (1972) found that swine diets containing 20% cassava leaf meal, which is relatively high in fibre, were greatly improved by increasing the energy level of the diet and adding methionine. Chick diets high in cassava meal have been improved to the level of a corn control by the addition of methionine (Olson et al. 1969), and those high in cassava leaf meal by increasing the metabolizable energy content as well as the methionine (Ross and Enriquez 1969).

A generalization is that diets containing a high level of cassava are likely to be deficient in methionine and cystine. These amino acids are proportionately low in cassava and, furthermore, it is a reasonable suggestion that cyanogens in the diet might increase the need for sulfur amino acids owing to a generally recognized need for cystine to participate in the detoxication of HCN.

The question remains: does chronic HCN toxicity from the ingestion of cyanogenic plants occur in domestic animals and, if so, what is the nature of the effect? Better controlled experiments than those cited above are needed to satisfactorily resolve this question.

## Neuropathic Role of Cyanide in Animals

Studies on the etiology of human ataxic neuropathy in Nigeria (Osuntokun et al. 1969) and in Tanzania (Makene and Wilson 1972) have led to the hypothesis that this condition is caused by chronic exposure to cyanide or cyanogens ingested in cassava. Whether a parallel situation might exist with domestic animals is not known. Experimental evidence from feeding trials designed to study neuropathological effects from cyanide is only available for rats and dogs. Martino (1935) reported that rats fed cassava roots developed neuromuscular symptoms. Lumsden (1950), Rose et al. (1954), Ibrahim et al. (1963), and Smith et al. (1963) subjected rats, and in one case dogs, to repeated sublethal doses of potassium cyanide and found lesions in the central nervous system but, in all cases, only in a small proportion of the animals

treated. Only for the experiments of Smith et al. in which very frequent small doses of potassium cyanide were used, can results be reasonably attributed to chronic rather than acute anoxic effects of cyanide. In a later study, Smith and Duckett (1965) following a similar low-level dosing procedure found an increased level of thiocyanate in the serum and confirmed their previous observations of degeneration of myelin in the central nervous system.

Certain similarities of ataxic neuropathy to neurolathyrism has led to interest in a possible relationship of chronic HCN toxicity to that given by the lathyrogens and neurotoxins, in general. The subject of lathyrism has been recently reviewed by Rao et al. (1969).

Lathyrism in animals is commonly considered to be of the osteo type. However, animals are susceptible to various neurotoxins contained in plants. An example is *Vicia sativa* which contains both a cyanogenic glycoside and the nitrile, $\beta$-cyanoalanine, and can produce neurotoxic symptoms in rats (Ressler 1962). Howell (1970) described other neurotoxins which can affect the nervous system of farm animals.

While the relationship among cyanogenic glycosides, HCN, and other cyano compounds in pathological effects in animals and humans may be coincidental, the subject deserves more study.

## Goitrogenic Role of Cyanide in Animals

Ekpechi et al. (1966) suggested that the incidence of human goitre in Nigeria is related to a chronic intake of HCN from cassava. Goitrogenic substances are present in many plants but have not generally been identified with cyanogenic glycosides. However, linseed meal which contains varying amounts of the cyanogenic glycoside, linamarin, gives goitrous lambs if fed to pregnant ewes (Care 1954). Also reported (Courrier and Cologne 1960) are lesions in the thyroids of rats fed linseed meal. Flux et al. (1956) and Butler et al. (1957) found no clear evidence of a goitrogenic effect from feeding white clover containing cyanogenic glycosides to sheep. However, in later studies, Flux et al. (1960, 1963) reported that lambs from ewes grazed on pastures containing cyanogenic clover, and in some cases the ewes, exhibited enlarged thyroids. The production of frank thyroid enlargement seemed to depend on the level of iodine intake relative to the HCN intake, not appearing if iodine intake was comparatively high. No deleterious effect on weight gains or lambing performance was observed in any of the sheep in these experiments.

It is possible that goitrogenic activity could arise from thiocyanate formed during detoxication of the HCN. Sihombing et al. (1971) found that administering thiocyanate to pigs decreased growth and produced enlarged thyroids. Langer (1966) found a similar effect with rats. Blakely and Coop (1949) administered potassium cyanide to sheep by rumen fistula and found a marked rise of thiocyanate in the serum and urine. It has already been noted that Flux et al. (1956) and Butler et al. (1957) observed increased plasma thiocyanate levels in sheep grazing on cyanogenic white clover.

Where goitrogenic effects can be attributed to thiocyanate or HCN acting directly on the thyroid gland or from some other compound, perhaps formed from thiocyanate or HCN, is not known. A number of plant substances can cause thyroid enlargement in animals, and it is of interest that where information is available such substances have in common a cyanide or closely related grouping. Rapeseed, especially the species *Brassica napus*, is a potent carrier of antithyroid compounds. The potentially goitrogenic compounds in the seed are glucosinolates (thioglucosides). These are carried over into the meal by-product from oil extraction, and can cause problems when the meal is used as a feed for livestock (Bowland et al. 1965). Analogous to the cyanogenic glycoside the glucosinolates must be hydrolyzed to release the toxic products and this occurs in the ground seed or meal if suitable enzymes are active. Several biologically active hydrolytic products may result depending, among other factors, on temperature, *p*H, age of seed, and also on the chemical nature of the aglycone moiety of the glucosinolates (van Etten et al. 1969). The best recognized products are thiocyanates, isothiocyanates and cyclized isothiocyanate, goitrin (5-vinyl-oxazolidine-2-thione). Figure 1 shows hydrolytic products of linamarin and typical products of the glucosinolates of rapeseed. The thiocyanates and particularly goitrin are goitrogenic. The activity of the former can be alleviated by supplementation of the diet with iodine, but the latter acts by direct interference with organification of the iodine (Lo and Hill 1971).

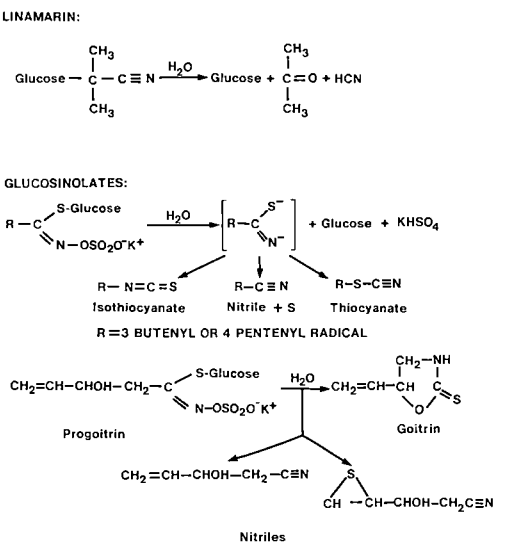


FIG. 1. Hydrolytic products of the cyanogenic glucoside, linamarin, from cassava and of the glucosinolates from rapeseed.

Saturated and unsaturated nitriles in which the cyanide grouping is not directly linked to sulfur can also result from the hydrolysis of rapeseed glucosinolates (Lo and Hill 1972a) and these substances, while rather unstable, are very toxic with an LD50 for rats of about one-eighth of that of goitrin. The severe growth depression exhibited by pigs, chicks, and rats consuming raw ground rapeseed could be attributed to nitriles rather than the known goitrogens. Heat-treated rapeseed is much less toxic and exhibits a much reduced goitrogenic action presumably because, like the cyanogenic plants, destruction of the hydrolyzing enzyme in the seed prevents, to a larger degree, subsequent release of the toxins. It is not known if the nitriles can in themselves act as goitrogens either directly or as intermediates in the synthesis of goitrogenic substances. The fate of ingested unhydrolyzed glucosinolates is not known but some hydrolysis of ingested glucosinolates can occur in the rat since Lo and Hill (1972a) found both nitriles and goitrin in the urine of rats which had been dosed with rapeseed meal prepared from enzyme-inactivated seed.

The less complicated chemical structure of the cyanogenic glycosides, as compared to the glucosinolates, probably precludes an equivalent complexity of hydrolysis products. However, the possibility of the formation of similar products during metabolism or of the occurrence of a yet unrecognized potentially toxic substance in cassava and plant materials in general should not be ignored. Recently, chlorogenic acid, a potent trypsin inhibitor (as judged by in-vitro tests), has been identified in the polyphenolic fraction of rapeseed (Lo and Hill 1972b).

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# Implications of Cyanide Toxicity in Animal Feeding Studies Using High Cassava Rations

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**Abstract** Studies on the chronic toxicity of cassava and/or added cyanide have been performed with rats and pigs. Methionine supplementation significantly improved body growth and feed conversion of animals fed cassava-based diets with or without added cyanide and led to an increased urinary excretion of thiocyanate. The improvement of the protein quality and the utilization of the methionine-sulfur in the detoxification processes appear to be the main reasons for the response to methionine supplementation. No gross thyroid lesions (goiter) have been observed on any of the rats fed either the control diet or cassava containing 150 mg of hydrocyanic acid per kilogram. A study on the methionine and iodine interaction is under progress and partial results are presented.

**Résumé** Les auteurs ont poursuivi, sur des rats et des procs, des études de toxicité chronique du manioc seule ou additionnée de cyanure. Un supplément de méthionine améliore sensiblement la croissance corporelle et l'efficacité de conversion des aliments chez des animaux soumis à un régime à base du manioc, avec ou sans addition du manioc, et entraîne l'élimination d'une plus grande quantité de thiocyanate dans l'urine. L'amélioration de la qualité des protéines et l'utilisation du soufre-méthionine dans le processus de désintoxication semblent être les raisons principales de la réponse au supplément de méthionine. Aucune lésion macroscopique de la thyroïde (goitre) n'a été observée chez les rats soumis soit au régime témoin soit à le manioc contenant 150 mg d'acide cyanhydrique par kilogramme. Les auteurs donnent les résultats préliminaires d'une étude en cours sur l'interaction de la méthionine et de l'iode.

CASSAVA (*Manihot* spp.) comprises a major source of food for a large portion of the world population and especially for those located in the tropical belt. Besides its important role in human nutrition, cassava also has been used as a feedstuff for livestock, and especially as an energy source for swine in the Philippines, Africa, and many areas of Latin America. Much of the published data on swine feeding has been reviewed (Maner 1972). Indications are that cassava is a good source of energy if used at levels of less than 30–40% in well-balanced diets. At higher levels slight growth depression and a decrease in efficiency of feed utilization are observed. However, this depression in pig performance can be overcome by the utilization of high-quality protein and the supplementation of the diets with adequate methionine.

## Protein Quality

Studies carried out by Portela and Maner (1971 unpublished data) clearly demonstrate the effect of methionine supplementation to growing pig diets based on cassava meal and soybean meal. Two replications of five pigs each per treatment

were used to study the effect of adding 0.1% or 0.2% DL-methionine to cassava-based diets containing 15.9% crude protein (Table 1). During the 42-day study the addition of methionine improved both growth and feed conversion (Table 2) although the differences in response were not statistically significant ($P > 0.05$).

TABLE 1. Composition of cassava diets for growing pigs (Portela and Maner 1971 unpublished data).

| Diet | Control group | Cassava-fed group |
|---|---|---|
| | % | % |
| Cassava meal | – | 56.0 |
| Soybean meal | 15.7 | 26.5 |
| Yellow maize | 79.3 | 12.5 |
| Bone meal | 3.5 | 3.5 |
| Vitamin Premix | 1.0 | 1.0 |
| Mineral Premix | 0.5 | 0.5 |
| Calculated protein (%) | 15.8 | 15.9 |

TABLE 2. Performance of growing pigs fed cassava-based diets with and without methionine supplementation (Portela and Maner 1971 unpublished data). Values with the same superscript are not statistically different ($P > 0.05$).

| Diet | Average daily gain[a] (*kg*) | Average daily gain (*kg*) | Feed/ gain |
|---|---|---|---|
| Corn–soybean | $0.74^{c}$ | 2.07 | $2.81$ |
| Cassava–soybean | $0.76^{bc}$ | 2.01 | $2.65^{ab}$ |
| Cassava–soybean–0.1% methionine | $0.83^{ab}$ | 2.03 | $2.46^{a}$ |
| Cassava–soybean–0.2% methionine | $0.82^{ab}$ | 2.03 | $2.49^{ab}$ |

[a]Two replications of five pigs each per treatment, 42-day experiment.

Similar results (Maner and Mesa 1971 unpublished data) were obtained with rats fed diets based on dried cassava meal and supplemented with casein. Supplemental levels of 0, 0.12, 0.17, 0.22, and 0.27% methionine were compared. A quadratic response in gain, feed efficiency and protein efficiency ratio (Table 3) was obtained. Performance was improved as the level of supplemental methionine was increased to 0.17% and decreased progressively at higher levels of supplementation.

A repetition of this study (Calderón and Maner 1972 unpublished data) produced similar results in that maximum performance during the 28-day trial was obtained when the level of methionine supplementation to the cassava meal based diet reached 0.17%. However, in contrast to the first trial, a depression in performance was not observed at higher levels of supplementation. Supplemental levels of 0.22 and 0.27% methionine supported performance not statistically different from that obtained with 0.17% methionine (Table 4).

## Cyanide Toxicity

Reports (Clark 1939; Montgomery 1965; Ekpechi et al. 1966; Oke 1968, 1969; Ermans et al. 1969; Osuntokun 1970; Osuntokun and Aladetoyinbo 1970; Delange and Ermans 1972; Thilly et al. 1972) have indicated that long-term consumption of cassava containing low levels of hydrocyanic acid (HCN) produce a chronic cy-

TABLE 3. Protein value of cassava protein with varying levels of methionine supplementation compared to casein protein. Five rats were used per treatment in this 16-day experiment (Mesa and Maner 1972 unpublished data).

| Diets[a] | Average gain (*g*) | Feed/ gain (*g*) | Protein efficiency ratio |
|---|---|---|---|
| Casein + Cassava | 28.5 | 5.1 | 2.35 |
| " " + 0.12% methionine | 33.7 | 4.4 | 2.63 |
| " " + 0.17% " | 41.9 | 3.9 | 2.91 |
| " " + 0.22% " | 39.9 | 4.2 | 2.73 |
| " " + 0.27% " | 31.6 | 4.8 | 2.52 |

[a]All diets contained 8.73% crude protein: 6.00% from casein and 2.73% from cassava.

TABLE 4. Effect of methionine supplementation to cassava–casein diets fed to growing rats (Calderón, Maner, and Gómez 1972 unpublished data).

| Per cent protein | Source of protein | Average[a] gain (*g*) | Feed/ gain | Protein efficiency ratio |
|---|---|---|---|---|
| 6.00 | Casein + Cassava starch | 13.3 | 10.9 | 1.52 |
| 6.00 | " " " + 0.12% DL-methionine | 47.0 | 5.6 | 2.95 |
| 8.63 | " " meal | 37.1 | 5.7 | 2.04 |
| 8.63 | " " " + 0.12% " | 56.4 | 4.7 | 2.49 |
| 8.63 | " " " + 0.17% " | 66.9 | 4.3 | 2.69 |
| 8.63 | " " " + 0.22% " | 67.3 | 4.2 | 2.76 |
| 8.63 | " " " + 0.27% " | 69.9 | 4.3 | 2.72 |
| 8.63 | " " starch | 53.4 | 4.5 | 2.60 |
| 8.63 | " " " + 0.18% " | 91.2 | 3.6 | 3.24 |
| 8.63 | " " " + 0.18% " | 56.7 | 4.1 | 2.79 |

[a]Twenty-eight-day feeding trial.

TABLE 5. Effect of HCN in cassava meal and fresh cassava on growth and urinary excretion of thiocyanate in rats; each value represents the average of four rats (Maner 1972 unpublished data).

| Days on expt | Diet | Average weight (*g*) | Thiocyanate excreted (*mg/day*) |
|---|---|---|---|
| 9 | Sucrose | 146 | 0.11 |
| | Cassava meal | 154 | 0.47 |
| | Fresh cassava | 177 | 3.69 |
| | Fresh cassava + $FeCl_2$(0.3%) | 174 | 3.72 |
| 14 | Sucrose | 174 | 0.12 |
| | Cassava meal | 194 | 0.49 |
| | Fresh cassava | 221 | 2.80 |
| | Fresh cassava + $FeCl_2$(0.3%) | 218 | 3.86 |
| 29 | Sucrose | 220 | 0.16 |
| | Cassava meal | 238 | 0.56 |
| | Fresh cassava | 271 | 4.50 |
| | Fresh cassava + $FeCl_2$(0.3%) | 288 | 5.02 |
| 70 | Sucrose | 344 | 0.20 |
| | Cassava meal | 333 | 0.56 |
| | Fresh cassava | 405 | 3.58 |
| | Fresh cassava + $FeCl_2$(0.3%) | 405 | 4.06 |

anide toxicity with resulting ataxic neuropathy and/or goiter. However, the effect appears to be complicated by other factors in addition to HCN and these factors appear to be predisposing for the development of the previously mentioned conditions.

Maner (1972 unpublished data) fed fresh chopped cassava containing 150 mg HCN/kg to growing rats for a period of 4 months to measure the effect HCN would have on performance, thyroid development, and the urinary excretion of thiocyanate (SCN). These parameters were compared to those of rats fed either sucrose or sun-dried cassava meal containing only 5 mg HCN/kg. Rats fed a protein supplement and fresh cassava gained weight at a significantly faster rate than those fed either the sucrose or the cassava meal-based diets (Table 5), even when the quantity of protein and dry matter was controlled for equal consumption by all groups. Average excretion of

TABLE 6. Effect of cyanide on body growth of rats in an 18-day experimental period (Calderón, Maner, and Gómez 1972 unpublished data).

| | Added cyanide (as KCN), *ppm* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 480 | 960 | 1600 | 2400 | 3200 | 4800 | 8000 |
| Initial no. rats | 5 | 5 | 5 | 5 | 5 | 5 | 4 | 4 |
| Final no. rats | 5 | 5 | 4 | 4 | 5 | 2 | 1 | 1 |
| Total wt gain, *g* | 27.9 | 24.4 | 18.5 | 14.6 | 11.5 | 2.6 | 4.1 | −9.1 |
| Feed consumed, *g* | 127.2 | 109.5 | 92.3 | 87.6 | 84.4 | 72.9 | 81.6 | 61.5 |

SCN in the urine was proportional to the quantity of HCN consumed. Significantly larger quantities (37-fold increase) were excreted by the rats consuming fresh cassava than by those fed sucrose-based diets, and those consuming dried cassava meal excreted five times as much SCN/day as those receiving the control sucrose diet. An autopsy showed no differences in thyroid size in any of the rats on the four different treatments.

Since no detrimental effects were observed when the fresh cassava containing 150 mg HCN was fed as part of a well-balanced and adequate diet, additional studies were undertaken to determine the effect higher levels of cyanide (–CN), added to the diet as potassium cyanide (KCN), would have on rat performance, serum and urinary SCN, methionine requirement, and goiter production.

Preliminary observations (Calderón, Maner, and Gómez 1972 unpublished data) suggest that 3200-ppm dosage or higher of KCN added to the diets was lethal for growing rats. One out of four rats survived in the groups fed a basal cassava starch–casein diet supplemented with 4800 and 8000 ppm cyanide, respectively, and two out of five when 3200 ppm was added. The total body weight gains in an 18-day experimental period declined as the levels of added cyanide increased from 0 to 8000 ppm (Table 6). Urine samples collected during the last 8 days of the experimental period were analyzed for SCN (Bowler 1944) and the results clearly showed an almost linear increase of urinary SCN excretion as the level of added cyanide was increased (Fig. 1). Plasma SCN concentration was also higher for rats fed the cyanide-supplemented diets (Fig. 2) than for rats fed the control diet (0 ppm $CN^-$), but the differences were not as pronounced as those observed in the SCN urinary excretion.

Although there is a great deal of information on the acute toxicity of HCN, relatively little is

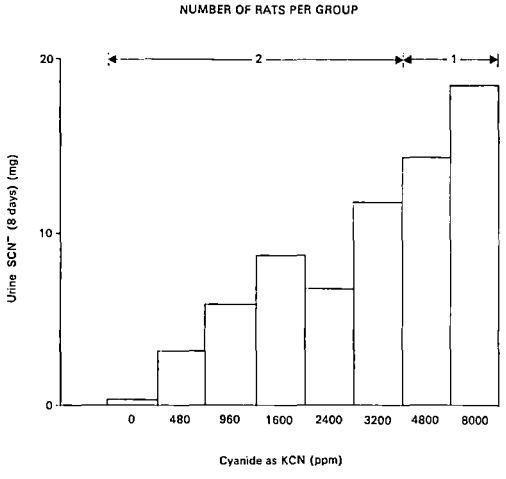


FIG. 1. Effect of cyanide on urinary thiocyanate excretion in rats.

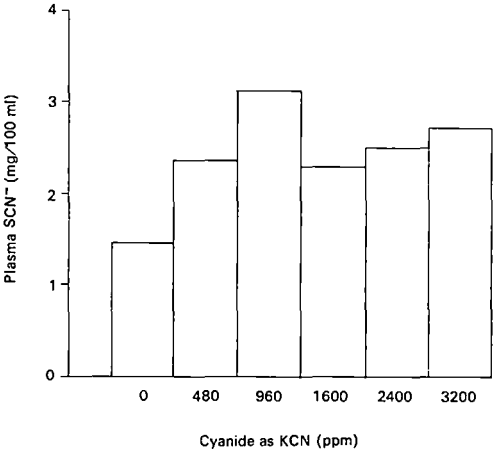


FIG. 2. Plasma thiocyanate concentration in rats fed varying levels of cyanide.

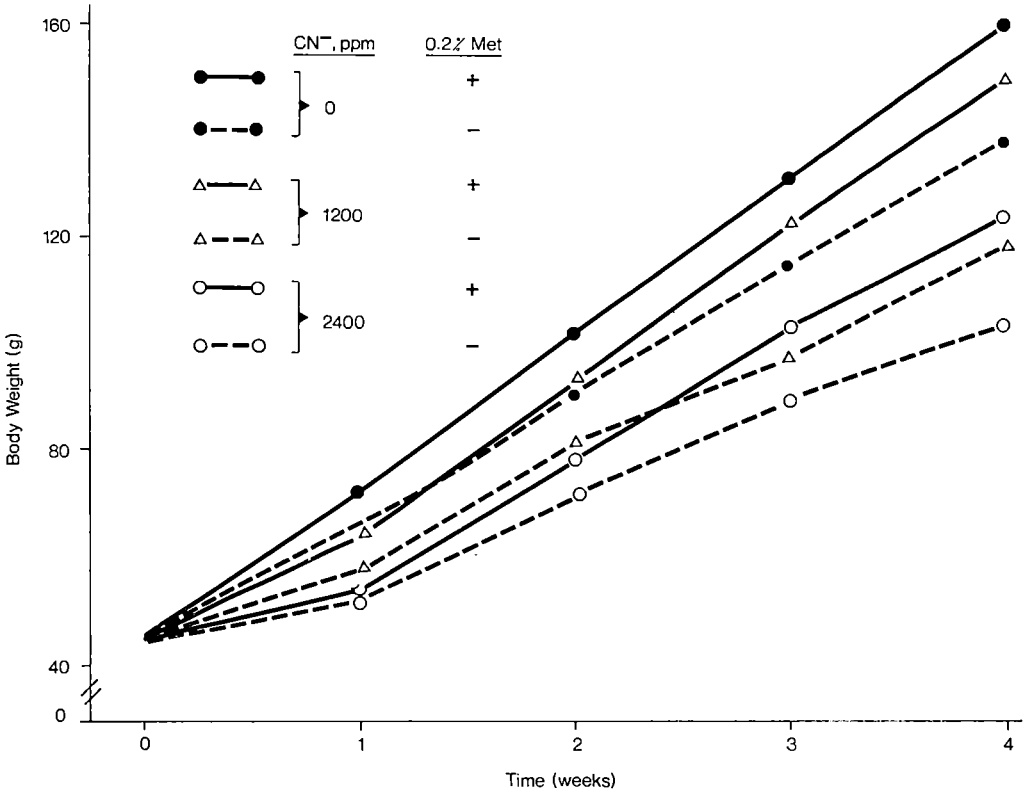


FIG. 3. Methionine and cyanide interaction on the body growth of rats (six rats per group).

known about the chronic effects derived from the continuous ingestion of sublethal amounts of cyanide. Our preliminary observations suggest that levels of added cyanide below 2400 ppm could be considered sublethal for rats. Previously cited (unpublished) observations with rats (Maner and Mesa) and pigs (Portela and Maner) have also demonstrated the beneficial effect of methionine supplementation to cassava diets, which was at first considered as the correction of a methionine deficiency per se occurring in the dietary protein but which more probably exerts its effect both by improving protein quality and as a readily available source of sulfur for cyanide detoxication through the thiosulfate–rhodanase system. Therefore, our subsequent studies (Calderón et al. unpublished data) using sublethal doses of added cyanide considered methionine supplementation as an additional variable. Since whole cassava roots (peel and pulp) are normally used in practical animal feeding, the experimental diets were formulated using cassava meal rather than cassava starch.

At all levels of added cyanide, rats fed the methionine-supplemented diets exhibited a significantly ($P < 0.01$) faster rate of growth than those fed unsupplemented diets (Fig. 3). Methionine supplementation improved feed consumption, and led to an increased urinary excretion of SCN as the level of added cyanide was augmented from 0 to 1200 and 2400 ppm, respectively (Table 7). Plasma SCN concentration did not follow the same pattern as the urinary excretion of SCN which increased as the level of cyanide and methionine were increased (Table 7). On autopsy none of the rats showed any indications of enlarged thyroids.

Reports in the literature (Delange et al., 1968; Ermans et al., 1969; Delange and Ermans, 1971; and Thilly et al., 1972) indicate that endemic goiter in the Congo and New Guinea cannot be explained solely on the basis of an iodine deficiency but may be complicated by dietary goitrogenic factors and especially by the large quantity of cassava consumed in the goitrous areas. The

TABLE 7. Interaction of methionine and cyanide supplementation to cassava meal diets in rats; six rats per treatment, 28-day experimental period (Calderón, Maner, and Gómez 1972 unpublished data).

| | Dietary variables | | | | | |
|---|---|---|---|---|---|---|
| | 0.2% methionine | | | 0% methionine | | |
| $CN^-$ ppm[3] | 0 | 1200 | 2400 | 0 | 1200 | 2400 |
| Total weight gain, *g* | 111.7 | 103.3 | 76.1 | 91.5 | 73.4 | 56.0 |
| Food consumed, *g* | 362.8 | 360.9 | 276.5 | 348.7 | 285.5 | 236.1 |
| Feed conversion | 3.3 | 3.4 | 3.7 | 3.8 | 3.9 | 4.2 |
| Total urine $SCN^-$, *mg*[a] | 68.1[b] | 87.5 | 116.0 | 43.7[b] | 62.3 | 63.5 |
| Plasma $SCN^-$, *mg/100 g* | 2.05[b] | 3.24 | 2.72 | 2.34[b] | 2.45 | 2.81 |

[a]$CN^-$ = cyanide as KCN; $SCN^-$ = thiocyanate.

[b]Mean of two rats; remaining values on the same lines represent means of three rats per group.

TABLE 8. Effect of methionine and/or iodine supplementation to cassava meal–casein diets fed to rats; partial results over 2-week experimental period using four rats per experiment. Average body weight of rats was 88 g (Gómez, Calderón, and Maner 1972 unpublished data).

| | Dietary variables | | | |
|---|---|---|---|---|
| | 0.2% methionine | | 0% methionine | |
| | Iodine | No iodine | Iodine | No iodine |
| Avg total wt gain, *g* | 65.5 | 64.6 | 37.0 | 27.5 |
| Avg food intake, *g* | 196.1 | 201.6 | 168.8 | 154.7 |
| *Avg total SCN urinary excretion* | | | | |
| Pre-experimental period (6 days), *mg* | 0.62 | 0.58 | 0.61 | 0.59 |
| First week experiment, *mg* | 4.31 | 3.55 | 2.61 | 2.41 |
| Second week experiment, *mg* | 4.76 | 4.25 | 3.62 | 2.82 |

goitrogenic activity of cassava was experimentally demonstrated in rats (Ekpechi et al. 1966), and further experimental evidence in humans (Delange and Ermans 1971) suggested that the ingestion of cassava grown in the goitrous area of Idjwi Island (Republic of Zaïre) reduces the thyroid iodine intake and increases the renal iodine excretion.

The prevalence of iodine deficiency in the tropics, and the wide use of cassava as a staple food in these areas, have led us to approach this problem in animal experiments. An experiment with rats was designed to study the interaction of supplemental methionine (0 and 0.2%) and iodine (addition of potassium iodide (KI) to, or its withdrawal from, the mineral mixture) on a cassava meal–casein diet. The partial results of the first 2 weeks of the experiment in four groups of rats kept in metabolic cages for urine collection are shown in Table 8. The effects of methionine supplementation on body growth of rats fed cassava meal-based diet are confirmed again. During a 6-day pre-experimental period, the rats were fed a pelleted-laboratory diet (Purina chows) and daily urine collections were analyzed for SCN. The daily urinary SCN excretion was consistently low throughout the entire pre-experimental period but the ingestion of the experimental diets (cassava meal–casein) brought about a rapid increase in the quantity of SCN excreted in the urine (Table 8). Despite the day-to-day variation, daily excretion of SCN in the urine followed a similar pattern for all the experimental groups. The total weekly SCN excretion (Table 8) was consistently lower in the absence of added methionine, and increased from the first to the second week in all the experimental groups, possibly as a consequence of the higher

TABLE 9. Analysis of the amino acid content of two samples of cassava, "Llanera" variety (Maner 1972 unpublished data).

| Amino acid | % of crude protein | |
|---|---|---|
| | Sample 1 | Sample 2 |
| Arginine | 17.10 | 12.90 |
| Histidine | .60 | .53 |
| Isoleucine | .77 | 1.04 |
| Leucine | 1.24 | 1.52 |
| Lysine | 1.54 | 1.56 |
| Methionine | –[a] | .33 |
| Cystine | .51 | –[a] |
| Threonine | .86 | 1.00 |
| Phenylalanine | .78 | .94 |
| Valine | 1.23 | 1.32 |
| Tryptophan | .50 | .50 |

[a]Non-detectable level.

food consumption. Further observations on thyroid and blood and tissue enzyme activities will be obtained at the end of the experimental period and published at a later date.

## Discussion

At least two reasons exist for the response to methionine supplementation of diets containing high levels of cassava. Cassava protein contains a very small quantity of sulfur-containing amino acids (Table 9) and when fed with casein or soybean meal, both deficient in methionine, there is a methionine deficiency per se which results from the combination of the two ingredients. The protein quality, therefore, is improved by the addition of crystalline DL-methionine. However, the methionine deficiency may be complicated by the utilization of the methionine-sulfur in the detoxication of HCN present in the cyanogenic glucosides of cassava. Since thiosulfate, and not methionine per se, is the required substrate for the formation of SCN by rhodanese (Himwich and Saunders 1948), methionine has to be metabolized so that methionine-sulfur would be available for detoxication purposes. However, SCN may be formed by a different mechanism such as the reaction of a persulfide group with cyanide, as proposed for the mechanism of xanthine oxidase inhibition by cyanide (Massey and Edmondson 1970).

Thiocyanate may be responsible for the goitrogenic activity of cassava as suggested by Delange and Ermans (1971). Pigs fed a corn–soybean meal diet supplemented with 0.5% potassium thiocyanate showed lower protein-bound iodine levels and heavier thyroids; however, higher incorporation of radioiodine in the thyroids was observed as compared to the parameters of pigs fed the same basal diet without SCN addition (Sihombing et al. 1971).

It therefore appears that methionine serves both to overcome a dietary deficiency of sulfur-containing amino acids and as a readily available source of labile sulfur for cyanide detoxication. The detoxication produces SCN that exerts a goitrogenic effect on the body that can cause thyroid hypertrophy especially in the absence of adequate dietary iodine. However, in the presence of adequate methionine and iodine, cyanide is without measurable effect on goiter production or nerve degeneration.

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# Cyanide and Human Disease

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WILSON, J. 1973. Cyanide and human disease, p. 121–125. *In* Chronic cassava toxicity: proceedings of an interdisciplinary workshop, London, England, 29–30 January 1973. Int. Develop. Res. Centre Monogr. IDRC-010e.

**Abstract** There is only circumstantial evidence linking human disease with chronic cyanide exposure, although there are many papers describing a variety of neuropathological changes in experimental animals.

The diseases which have so far been considered to result from abnormal detoxication of cyanide (mostly derived from tobacco smoke) are: retrobulbar neuritis in pernicious anaemia; tobacco amblyopia; subacute combined degeneration of the cord in vitamin $B_{12}$ deficiency; Leber's hereditary optic atrophy; and dominantly inherited optic atrophy.

In the first condition the abnormal sensitivity to cyanide may be conditioned by acquired vitamin $B_{12}$ deficiency. In Leber's disease an inborn metabolic error may prevent mobilisation of sulfur-containing substrates for thiocyanate formation. In dominantly inherited optic atrophy, the metabolic basis is not known, but there is an abnormally high concentration of cyanocobalamin present in the plasma of most patients.

While it is acknowledged that in the ataxic neuropathy of West Africa the aetiology is probably multifactorial, heavy exposure to cyanide or cyanogens from cassava may be particularly damaging in the nutritional context of lack of protein and riboflavin.

**Résumé** Nous n'avons que des preuves indirectes que l'exposition chronique au cyanure cause des maladies chez l'homme, bien que plusieurs travaux décrivent les changements neuropathiques provoqués expérimentalement chez des animaux.

Les maladies qui, jusqu'à présent, ont été considérées comme résultant de la désintoxication anormale du cyanure (provenant surtout de la fumée de tabac) sont: névrite rétrobulbaire associée à l'anémie pernicieuse; amblyopie causée par le tabac; dégénération combinée subaiguë de la corde associée à une déficience de vitamine $B_{12}$; atrophie optique héréditaire de Leber; et atrophie optique à caractère héréditaire dominant.

Dans le premier cas, la sensibilité anormale au cyanure peut être conditionnée par une déficience acquise de vitamine $B_{12}$. Dans la maladie de Leber, un dérangement métabolique inné peut empêcher la mobilisation de substrats contenant du soufre vers la formation de thiocyanate. Dans le cas de l'atrophie optique à caractère héréditaire dominant, on en ignore le principe métabolique, mais la plupart des patients possède une concentration anormalement élevée de cyanocobalamine dans le plasma.

Tout en reconnaissant l'aspect multifactoriel probable de l'éthiologie de la neuropathie ataxique en Afrique occidentale, l'exposition chronique au cyanure ou à des cyanogènes provenant du manioc peut être particulièrement dommageable dans le contexte nutritionel d'une déficience de protéines et de riboflavine.

EXAMINATION of the medical literature for direct evidence of disease resulting from chronic cyanide exposure is disappointing.

The heavy exposure to volatile cyanide that still sometimes occurs in the metal-refining industry, in metal-cleaning, and in certain photographic processes is variously reported as causing headaches, weight loss (with curious preservation of appetite), and lassitude. It has also been suggested that some Chilean ore-refining workers have developed a clinical picture resembling Parkinson's Disease, but it is not clear if the patients in question had, in fact, been overcome and partially asphyxiated. The bulk of the evidence I will review is, therefore, indirect and highly circumstantial. It is based largely on demonstrating an association between certain diseases and presumed cyanide exposure either to free hydrogen cyanide (in tobacco smoke) or to cyanogens (in foods). Under normal circumstances, cyanide from these sources appears to be virtually harmless, reflecting the efficiency of the metabolic pathways of detoxication.

Certain experimental studies in the 1930s and 1940s explored the possibility that disturbances in cyanide metabolism might cause human demyelinating disease, notably multiple sclerosis, and as models these studies were not without merit (e.g. Ferraro 1953; Meyer 1933; Rubino 1935; Jedlowski 1937; Jervis 1937; Hurst 1940, 1942; Hicks 1950; Lumsden 1950). Various focal and diffuse lesions of both white and grey matter were produced in a number of different species, but it is extremely difficult to be sure if these were the direct or indirect effects of cyanide. In some of the experiments animals were manifestly asphyxiated, whereas in others relatively large amounts of unbuffered alkaline salts were given parenterally. The interest in cyanide toxicity in multiple sclerosis has never been revived.

In the late 1950s when it was recognised that visual failure in vitamin $B_{12}$ deficiency occurred predominantly in males, and they were invariably smokers (Heaton et al. 1958), Wokes (1958) suggested that in view of the suspected metabolic interrelationship between cyanide and vitamin $B_{12}$, the causal factor in smoking was probably cyanide. This hypothesis was extended to include tobacco amblyopia, a clinically similar syndrome, in which plasma vitamin $B_{12}$ levels are significantly lower than normal, but are not usually in the grossly deficient range (Smith 1961).

Subsequently, Wilson et al. (1971) showed that in the latter condition there is an abnormal increase in the proportion of cyanocobalamin in plasma but not to the extent suggested by Smith (1961). Chisholm et al. (1967) demonstrated that not only do symptoms remit on treatment with large doses of vitamin $B_{12}$, but that hydroxocobalamin is therapeutically much superior to cyanocobalamin. It has been suggested that tobacco amblyopia and retrobulbar neuritis in pernicious anaemia may result either from a relative block in cyanide detoxication from absolute vitamin $B_{12}$ deficiency, leading to a damaging accumulation of cyanide (Wilson and Matthews 1966), or from the conversion of one of the physiologically active forms of vitamin $B_{12}$ to a physiologically inactive form (Smith 1961). I will discuss the relative merits of these hypotheses later.

Concurrently with the interest in the role of cyanide in the pathogenesis of visual disturbance in vitamin $B_{12}$ deficiency, I became interested in a rare neurological disease known as Leber's hereditary optic atrophy. This curious malady presents as a more or less severe visual failure occurring acutely or subacutely in males, usually in their late teens. Damage to the central fibres in the optic nerve is usually severe and the blindness permanent. In some patients, there is evidence of other diffuse damage to the central nervous system. Eighty-five percent of European and American patients with this condition are male, and this observation together with the age of onset suggested that smoking might be an environmental factor precipitating the clinical manifestations of an inborn metabolic error (Wilson 1963).

This prompted a study of thiocyanate concentrations in body fluids, revealing that the increment in thiocyanate concentration seen in normal smokers compared with non-smokers, is reduced in Leber's disease, and suggested an abnormality in the conversion of cyanide to thiocyanate (Wilson 1965) (Fig. 1). Moreover, more recent studies of plasma cobalamins showed an abnormal increase in plasma cyanocobalamin concentration not only in patients, but also in clinically affected carriers (Wilson et al. 1971). Not all patients are smokers. One of my cider-loving patients developed his symptoms quite dramatically when he went holidaying in a Sussex village deliberately chosen because it is the place where so-called vintage cider is brewed. His bibulous vacation terminated abruptly and having travelled there on a motor scooter he had to return to London by train be-

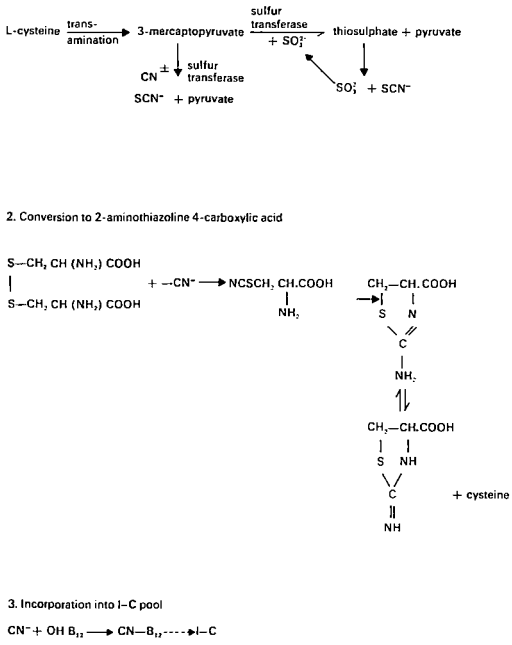



FIG. 1. Conversion of cyanide to thiocyanate; conversion to 2-aminothiazoline 4-carboxylic acid; incorporation into 1-C pool.

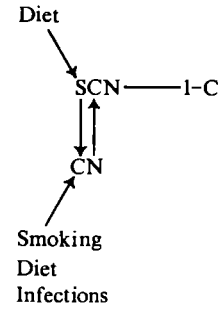
cause he could not see. Another patient, also a non-smoker, developed her symptoms following a flare-up in a chronic urinary-tract infection due to *Pseudomonas pyocyanea*, a microorganism known to produce large amounts of free cyanide.

Some of our control samples for our study of cobalamins in Leber's disease were obtained from patients with another hereditary ophthalmological disease—dominantly inherited optic atrophy—which is clinically and genetically quite distinct from Leber's disease. This disease usually manifests itself first about the age of 5 years, and is not obviously associated with tobacco smoking. To our surprise, cyanocobalamin levels were also abnormally elevated compared with normal subjects, and this was independent of smoking habits (as in Leber's disease). It is possible, therefore, that this disease may also represent the clinical effects of an inborn metabolic error of cyanide and/or vitamin $B_{12}$ metabolism.

## Vitamin $B_{12}$ Deficiency

Human vitamin $B_{12}$ deficiency usually occurs as the result of a failure of vitamin $B_{12}$ absorption from the gut. The principal manifestation is a macrocytic anaemia of varying severity, known as pernicious anaemia, usually accompanied by a conspicuous loss of appetite. In a small minority of patients there is evidence of diffuse disease of the spinal cord and peripheral nerves, known as subacute combined degeneration (SACD). There is an interesting dissociation between the degree of anaemia and severity of the neurological disease. Another interesting feature is the differing sex-incidence.

The male to female ratio in patients with uncomplicated anaemia is 1:2.5, but in patients with SACD the ratio is 1:1.8. The relative excess of males is made up of smokers. In an attempt to explain these observations—the dissociation between the degree of anaemia and SACD, and the excess of smokers with SACD—Langman and I proposed what I still consider is a very neat hypothesis (Langman and Wilson 1966). We suggested that the metabolic equilibrium between thiocyanate and cyanide maintained normally thus:




is deranged by vitamin $B_{12}$ deficiency, vitamin $B_{12}$ being essential for the incorporation of cyanide into the single carbon unit metabolic pool. Two further factors operated, according to our hypothesis. First, the reduction in appetite diminishes the amount of thiocyanate ingested (e.g. milk, beer, *Brassica*) and, second, and in parallel, reduces the intake of dietary folate, which in turn increases the severity of the anaemia. This in turn affects the cyanide concentration in the plasma indirectly because we presumed that the activity of the red-cell enzyme, so-called "thiocyanate oxidase" (Goldstein and Rieders 1951, 1953) was dependent on total red-cell mass. We now know that thiocyanate oxidase activity is a property of free haemoglobin and its derivatives.

We studied this relationship prospectively, and

TABLE 1. Mean plasma thiocyanate and haemoglobin concentration (data from Wells et al., Brit. Med. J., 1972).

| Clinical group | Sex M | Sex F | Smokers or non-smokers | Mean plasma thiocyanate concentration ($\mu mole/100\,ml$, $\pm$SD) | Mean haemoglobin concentration ($g/100\,ml$, $\pm$SD) |
|---|---|---|---|---|---|
| 1 Uncomplicated pernicious anaemia | 9 | 28 | NS | $3.9 \pm 1.9$ | $6.9 \pm 2.2$ |
| | 5 | 6 | S | $7.9 \pm 5.0$ | $7.4 \pm 1.3$ |
| 2 Dementia and vitamin $B_{12}$ deficiency | 4 | 8 | NS | $5.4 \pm 2.0$ | $9.3 \pm 2.8$ |
| 3 Subacute combined degeneration | 3 | 7 | NS | $5.0 \pm 1.8$ | $11.3 \pm 2.5$ |
| | 5 | 6 | S | $6.0 \pm 3.3$ | $10.6 \pm 2.9$ |
| 4 Folate deficiency | 0 | 10 | NS | $2.4 \pm 1.2$ | $7.0 \pm 2.6$ |
| 8 Combined controls (groups 5, 6, and 7)[a] | 25 | 8 | NS | $4.4 \pm 1.7$ | – |
| | 34 | 0 | S | $9.9 \pm 4.0$ | – |

[a]Mean plasma thiocyanate concentrations respectively 4.2, 4.6, and 4.3 $\mu$mole/100 ml in non-smoking normal controls (group 7), coronary thrombosis patients (group 6), and elderly medical controls (group 5). Concentrations respectively 10.1, 9.6, and 9.9 $\mu$mole/100 ml for smokers. Statistical comparison of mean thiocyanate concentrations ($t$-test): Group 8 (NS) vs group 4 (NS) $P < 0.001$; group 8 (S) vs group 1 (S) $P > 0.05$; group 8 (S) vs group 3 (S) $P < 0.01$; group 1 (NS) vs group 3 (NS) $P > 0.05$.

the results were published recently (Wells et al. 1972; Table 1).

These results do indeed support the suggestion that thiocyanate intake parallels folate intake, but go no further. If our hypothesis is correct, the data suggest that variation in red-cell mass is more important than variation in thiocyanate concentration in determining cyanide concentration. Analysis of all our data does, however, show that there is a direct relationship between haemoglobin concentration and thiocyanate level. The one outstanding observation in this study which confirmed our earlier studies was the relationship between smoking and SACD.

The crucial evidence lacking from this and all other laboratory studies is data on cyanide concentrations. Unfortunately, none of the techniques of plasma cyanide assay is really satisfactory for the ultra microassay of free cyanide. All techniques depend on an initial deproteinisation and do not therefore differentiate bound from free cyanide, and the latter is probably metabolically active. Cyanide ions are so very active chemically and have a potent inhibitory effect on such a wide variety of enzymes, that in my view it would seem likely that the pathological consequences are due to direct enzyme inhibition.

## Summary

It is acknowledged that this evidence for the role of cyanide in human neurological disease is still very indirect but no more circumstantial, perhaps, than those studies relating smoking to cancer of the lung. Since much of the evidence, however, is concerned with a relationship to smoking (except in the case of dominantly inherited optic atrophy), it could be argued that the abnormalities in cyanocobalamin concentration are merely an indirect index of excessive tobacco smoke exposure. I think this is an unlikely explanation. Likewise, I do not think the hypothesis of vitamin $B_{12}$ inactivation is tenable since, even in the heaviest smoker with either vitamin $B_{12}$ deficiency or Leber's optic atrophy, there is no chromatographic evidence of a preponderance of cyanocobalamin, much less total conversion. The biggest stumbling-block at present is the failure to demonstrate either an absolute increase in the concentration of free cyanide in body fluids or an enzyme block in those genetically determined diseases where it is reasonable to expect one.

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# Ataxic Neuropathy Associated with High Cassava Diets in West Africa

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**Abstract** In Nigerians, a syndrome referred to as tropical ataxic neuropathy comprises lesions of the skin, mucous membranes, optic and auditory nerves, spinal cord, and peripheral nerves. The disease affected all age groups, but was rare in the 1–10-year-olds. Familial cases accounted for two-fifths of the patients, but there was no evidence of genetically determined predisposition. Patients subsisted mainly on a cassava diet.

The thiocyanate content of food items commonly eaten by Nigerians is low, whereas the cyanide content, especially of cassava derivatives, is high.

Plasma levels of thiocyanate, cyanide, and urinary thiocyanate excretion were high in patients. The levels fell when patients were fed on low-cassava hospital diet and rose again when the patients reverted to cassava meals. Levels of free cyanide in blood were raised. Sulfur-containing amino acids were absent in the plasma in 60% of the patients and reduced greatly in others. In patients, the level of serum cyanocobalamin (probably a product of cyanide detoxication) as measured by chromatography and bioautography was high. Total serum $B_{12}$ (microbiological assay) was normal. Normal urinary excretion of methylmalonic acid before and after valine loading excluded abnormal $B_{12}$ metabolism.

Epidemiological studies showed correlation of prevalence of the disease with intensity of cassava cultivation, frequency of cassava meals, and plasma thiocyanate levels. The prevalence of the disease in one high cassava-eating village was 3% and in the 50–60-year-old age group was 8%. Cassava farmers and processors appeared to have the highest risk of developing the disease.

The neuropathology of the disease would be compatible, with a few exceptions, with the effects of chronic cyanide intoxication.

The prevalence (2–5%) of goitre in patients was higher than in the population and appeared to be related to cassava diet and high plasma thiocyanate level.

Detailed studies to exclude dietary deficiencies, other intoxications, and metabolic derangements showed only low plasma levels of riboflavin and caeruloplasmia and low urinary excretion of riboflavin. The effects of riboflavin deficiency may combine with those of chronic cyanide intoxication.

Therapeutic trials with hydroxocobalamin, riboflavin, and cystine tablets yielded no beneficial effects.

**Résumé** Un syndrome désigné sous le nom de neuropathie atoxique tropicale se manifeste chez les Nigériens par des lésions de la peau, des muqueuses, des nerfs optique et auditif, de la moëlle épinière et des nerfs périphériques. La maladie affecte tous les groupes d'âge, mais est rare chez les moins de 10 ans. Les deux cinquièmes des patients sont des cas familiaux, mais nous n'avons pas de

preuve qu'il s'agisse de prédisposition héréditaire. Les patients ont un régime alimentaire surtout à base du manioc.

La teneur en thiocyanate de la nourriture ordinaire des Nigériens est faible, alors que la teneur en cyanure, surtout des dérivés du manioc, est élevée.

Les niveaux de thiocyanate et de cyanure sont élevés dans le plasma des patients, de même que celui du thiocyanate dans l'urine. Ces niveaux baissent lorsque les patients sont soumis au régime hospitalier, faible en manioc, et s'élèvent de nouveau avec un retour aux repas du manioc. Le niveau de cyanure sanguin libre s'élève. Les aminoacides contenant du soufre sont absents du plasma de 60% des patients et sont considérablement réduits chez les autres. Le niveau de cyanocobalamine plasmatique (probablement un produit de désintoxication du cyanure), mesuré par chromatographie et bioautographie, est élevé. La teneur en vitamine $B_{12}$ totale du sérum (essai microbiologique) est normale. Une excrétion normale d'acide méthylmalonique avant et après charge d'uraline exclut l'hypothèse d'un métabolisme anormal de la vitamine $B_{12}$.

Des études épidémiologiques démontrent une corrélation entre la fréquence de la maladie et l'intensité de la culture du manioc, la fréquence des repas à base du manioc et les niveaux de thiocyanate dans le plasma. L'incidence de la maladie dans un village où la consommation du manioc est élevée est de 3%; elle est de 8% chez le groupe d'âge de 50 à 60 ans. Le risque de maladie semble plus grand chez les personnes engagées dans la culture et la transformation du manioc.

La neuropathologie de la maladie est compatible, à peu d'exceptions près, avec les effets d'une intoxication chronique au cyanure.

La fréquence (2–5%) du goitre chez les patients est plus élevée que dans l'ensemble de la population et semble liée à la présence du manioc dans le régime alimentaire et au niveau élevé de thiocyanate dans le plasma.

Des études détaillées, excluant les déficiences alimentaires, les autres intoxications et les dérangements métaboliques, démontrent un faible niveau seulement de riboflavine dans le plasma, une caeruloplasmie et une basse excrétion urinaire de riboflavine. Les effets d'une carence en riboflavine peuvent se combiner avec ceux de l'intoxication chronique au cyanure.

Des essais thérapeutiques à l'aide d'hydroxycobalamine, de riboflavine et de cystine en comprimés ont été sans succès.

SYNDROMES comprising lesions of the skin, mucous membranes, and the nervous system, and usually attributed to dietary deficiency and/or toxin, have been described in communities with low standards of nutrition, especially in the tropics or in prisoner-of-war camps (Spillane 1947; Money 1959; Montgomery et al. 1964; Osuntokun 1968). It is unlikely that the specific etiological factors are the same or occur in the same proportion in every country where these syndromes have been reported. Similar clinical syndromes may be the result of different intoxications, dietary deficiencies, and other pathogenetic lesions as demonstrated by the peripheral neuropathies. This is not surprising as the nutritional welfare of the nervous system is dependent on energy derived mainly from carbohydrate (which it does not store) and a variety of complex enzyme systems which govern and control the use of this energy. The same biochemical lesion may be due to different deficiencies and intoxications. Thiamine deficiency causes impaired oxidative decarboxylation of the keto acids, and deficiencies of nicotinic acid and riboflavin impair the function of nicotinamide-adenine dinucleotide (NAD), nicotinamide adenine dinucleotide phosphate (NADP), flavin mononucleotide (FMN), and flavin adenine dinucleotide (FAD). Iron deficiency impairs function of the cytochrome system. All these will produce a similar biochemical result as cyanide-induced inhibition of cytochrome oxidase by impairing transfer of electrons to molecular oxygen and retarding oxidative metabolism. One substance may influence the effects of another. Deficiency of vitamin $B_{12}$ may enhance the toxic effects of hydrogen cyanide. The decyanation of cyanocobalamin to the physiologically active forms—hydroxocobalamin and coenzyme $B_{12}$—requires NAD, FMN, and FAD (Cima, et al. 1967). Thiamine is rendered inactive by hydrogen cyanide and sulfite which may be derived from the reaction of cyanide with thiosulfate (Clark 1936; Goldsmith 1964; Spillane 1969).

Being fully aware of these possibilities during the past 8 years, we have carried out in Ibadan, Nigeria, studies to identify the etiological factors in the disease known as tropical ataxic neuropathy

(TAN). In Nigerians, the essential neurological components of the disease are myelopathy, bilateral optic atrophy, bilateral perceptive deafness, and polyneuropathy. In about 35% of the patients stomatoglossitis is present. Motor neurone disease, Parkinson's disease, cerebellar degeneration, psychosis, and dementis have also been found in association with the disease (Asuni 1967; Osuntokun 1968, 1969). Our findings support earlier suggestions by Moore (1934a, b, 1937), Clark (1935, 1936), and Oluwole (1935) that an important factor in the etiology of TAN in Nigerians is chronic cyanide intoxication from a cassava diet.

## Cassava Diet and Neuropathy

I have studied over 360 Nigerians who suffer from TAN. The disease affects males and females equally and all age groups, but occurs only rarely in children under 10 years. The peak incidence is in the 40–50-year-old group.

A history of almost total dependence on a monotonous diet of cassava derivatives obtained in all patients. Occasional dietary supplements included yam, maize, rice, vegetables, and animal protein.

Only about 5% of the patients smoked a few cigarettes (1 to 3) per day or drank (and only occasionally) alcoholic beverages.

In 41% of the patients, at least someone else in the family was "similarly affected." In all cases the afflicted members of the family had all lived in the same environment and had a similar dietary history. Analysis of the relationship in the affected members showed no evidence of a genetically determined predisposition. The families were poor and members lived communally. Multiple conjugal cases in which husband, wife, and co-wives (in polygamous homes) were affected accounted for 65% of the family cases. Daughters and sons were affected in about 10% of these families. Wives were more frequently afflicted than husbands.

The cyanide and thiocyanate contents of various foodstuffs commonly eaten by the patients and other Nigerians were estimated (Table 1). The cyanide content of gari, eba, and purupuru, which are derivatives of cassava, is higher than that of other food items. In some areas of southern Nigeria where the disease exists in almost epidemic proportions, purupuru, which contains the highest concentration of cyanide, is commonly eaten. The high cyanide content of purupuru is due to the mode of preparation from cassava roots. If a man consumes 3 kg of purupuru per day, the total cyanide intake per day is about 50 mg. (The lethal dose of cyanide for man is 60 mg.) Deaths following cassava meals are occasionally reported in the Nigerian press. Intragastric release of cyanide occurs after cassava meals (Osuntokun and Aladetoyinbo 1970). The thiocyanate content of food items commonly eaten by Nigerians is low. Foodstuffs containing high concentrations of thiocyanate, such as milk, beer, and vegetables of the *Brassica* family including cabbage, are only rarely consumed (and then in small quantity) by Nigerians who suffer from TAN.

TABLE 1. Cyanide content (released during hydrolysis with oxygen in HCl at 37°C for 2 h) and thiocyanate concentrations of common items of food eaten by Nigerians.

| | Thiocyanate[a] (*μmoles/g*) | Cyanide[a] (*μmoles/g*) |
|---|---|---|
| Gari/Eba | 0.0042 | 0.027 |
| Purupuru | 0.0073 | 0.10 |
| Yam | 0.0033 | 0.0006 |
| Rice | 0.0037 | 0.0006 |
| Plantain | 0.0039 | 0.0009 |
| Beans | 0.0035 | 0.0008 |

[a]Thiocyanate was determined by the Aldridge (1944) method on deproteinised samples, and cyanide was determined spectrophotometrically by the Boxer and Richards (1950) method after concentration in an alkali trap.

## Assessment of Chronic Cyanide Intoxication

Cyanide is detoxicated in the body largely by conversion to thiocyanate, catalysed mainly by rhodanese present in most tissues (and in the highest concentration in the liver) in man, and to a lesser extent by mercaptopyruvate:cyanide sulfur transferase. The major substrates for this conversion are thiosulfate and 3-mercaptopyruvate, derived mainly from cysteine. Cyanide may also react spontaneously in vitro with cystine to form 2-aminothiazoline-4-carboxylic acid or its stochiometric isomer, 2-imino-4-thiazolidine carboxylic acid. Cyanide by interaction with hydroxocobalamin is incorporated into the 1-carbon metabolic

TABLE 2. Plasma thiocyanate and cyanide and urinary thiocyanate in patients with TAN, and normal Nigerians (members of staff, University College, Ibadan).

| | Plasma thiocyanate (*μmoles/100 ml*): Mean ± SE | Plasma cyanide (*μmoles/100 ml*): Mean ± SE | Urinary thiocyanate (*μmoles/kg body wt/24 h*): Mean ± SE |
|---|---|---|---|
| Patients[a] | 11.3 ± 0.2 ($n = 364$) | 0.1 ± 0.004 ($n = 108$) | 2.4 ± 0.1 ($n = 67$) |
| Normal Nigerians | 2.9 ± 0.02 ($n = 106$) $P < 0.001$ | 0.03 ± 0.002 ($n = 106$) $P < 0.001$ | 0.6 ± 0.05 ($n = 40$) $P < 0.001$ |

[a]Determinations were made within 48 h of admission.

TABLE 3. Decrease in plasma thiocyanate and cyanide and urinary thiocyanate in 69 patients with TAN.

| | On admission[a] | After 6 weeks hospital diet[a] |
|---|---|---|
| Plasma thiocyanate (*μmoles/100 ml*): Mean ± SE | 11.1 ± 0.45 | 2.8 ± 0.13 |
| Plasma cyanide (*μmoles/100 ml*): Mean ± SE | 0.097 ± 0.0045 | 0.026 ± 0.0014 |
| Urinary thiocyanate (*μmoles/kg body wt/24 h*): Mean ± SE | 2.42 ± 0.12 | 0.69 ± 0.031 |

[a]The differences between the concentration on admission and after 6 weeks on hospital diet are statistically significant ($P < 0.001$ for each variant).

TABLE 4. Plasma thiocyanate and cyanide in 35 patients with TAN.

| | Plasma thiocyanate (*μmoles/100 ml*) Mean ± SE | Plasma cyanide (*μmoles/100 ml*) Mean ± SE |
|---|---|---|
| Within 48 h of admission | 11.9 ± 0.8 | 0.11 ± 0.006 |
| After 6 weeks hospitalization (hospital diet) | 2.8[a] ± 0.3 | 0.025[a] ± 0.002 |
| Twelve weeks after discharge (cassava meals) | 12.8 ± 0.9 | 0.11 ± 0.07 |

[a]The difference between this figure and the one above and below is significant at the $P < 0.0001$ level. The differences between determinations made upon admission and 12 weeks after discharge are not significant.

pool, with the formation of cyanocobalamin and probably thiocyanate as well. Chronic exposure to cyanide can therefore be assessed by estimation of cyanide and thiocyanate (detoxication product of cyanide) in biological fluids, and alteration in amino acid composition of plasma. Qualitative changes in the plasma cobalamins may also reflect chronic cyanide intoxication. The levels of plasma thiocyanate, cyanide, and urinary excretion of thiocyanate were raised in patients (Table 2). These levels fell (Table 3) (and were sometimes accompanied by clinical or symptomatic improvement) when patients were fed a hospital diet containing cassava meals only twice a week. The levels rose again when the patients returned to a cassava diet after discharge from the hospital (Table 4).

Free cyanide as distinct from bound cyanide occurred in higher concentration in the blood of patients compared with normal Nigerians (Table 5).

The activity of the enzyme rhodanese in the liver of patients was normal (Osuntokun and Aladetoyinbo 1970).

Sulfur-containing amino acids (methionine, cysteine, and cystine) were absent in the plasma in 60% of the patients and the concentration was greatly reduced in the others. Plasma concentrations of some of the essential amino acids (e.g. valine, leucine, phenylalanine) were reduced, but on the other hand the concentrations of other essential amino acids such as isoleucine and threonine were within normal limits. The qualitative and quantitative changes in the plasma amino acids in Nigerian patients with TAN are unlike those found in kwashiorkor, the classical disease

TABLE 5. Free and bound cyanide in patients with TAN and controls.

| | Free cyanide in blood[a,b] (*μmoles/100 ml*) Mean ± SE | Bound cyanide in plasma[a] (*μmoles/100 ml*) Mean ± SE |
|---|---|---|
| Patients ($n = 37$) | 0.051 ± 0.003 | 0.108 ± 0.003 |
| Controls ($n = 40$) | 0.031 ± 0.003 | 0.051 ± 0.004 |

[a]The differences in the concentrations of free cyanide in blood and bound cyanide in plasma are statistically significant ($P < 0.01$).

[b]Free cyanide in blood was measured by a modification of the Boxer and Richards (1950) method, and described in detail elsewhere (Osuntokun et al. 1970a).

TABLE 6. Concentration of vitamin $B_{12}$ using the microbiological assay methods of Matthews (1962).

| | Serum $B_{12}$ ($10^{-12}$ g/ml; mean ± SE) | |
|---|---|---|
| | With cyanide extraction | Without cyanide extraction |
| Patients ($n = 320$) | 1764 ± 76 | 1141 ± 57 |
| Controls ($n = 114$) | 1430 ± 94 | 575 ± 44 |

of protein-calorie deficiency (Osuntokun et al. 1968; Osuntokun 1972). It seems likely that in some of the patients there was a conditioned deficiency (due to chronic cyanide intoxication) of sulfur-containing amino acids.

## Vitamin $B_{12}$ Metabolism

The concentration of vitamin $B_{12}$ as measured by microbiological assays (using *Lactobacillus leichmanii*) by the method of Matthews (1962) was normal in patients (Table 6). Serum $B_{12}$ levels were often very high in patients and controls. These were not due to renal, hepatic, and haematopoietic diseases, but were believed due to excessive protein binding (Fleming 1968). Microbiological assays of vitamin $B_{12}$ with and without cyanide extraction, interpreted according to Smith (1961), suggest that serum cyanocobalamin (probably a product of cyanide detoxication) is high in the Nigerian patients and that serum hydroxocobalamin is reduced (Monekosso and Wilson 1966; Osuntokun 1968, 1969). Lately it has been shown that such an interpretation of cyanide extraction of $B_{12}$ in microbiological assays bears no meaningful correlation with the various forms of vitamin $B_{12}$, i.e. methylcobalamin ($MeB_{12}$), hydroxocobalamin ($OHB_{12}$), cyanocobalamin ($CNB_{12}$), and deoxyadenosylcobalamin ($CoB_{12}$). It appears that cyanide extraction in microbiological assay of vitamin $B_{12}$ does not give any useful information about qualitative and quantitative assessment of plasma cyanocobalamins (Matthews and Wilson 1971; Matthews and Linnell 1971). We have recently estimated plasma and tissue individual cobalamins by chromatography and bioautography as described by Linnell et al. (1969a, b, 1971) and Matthews and Linnell (1971). Table 7 shows that cyanocobalamin ($CNB_{12}$) occurred in higher concentration in the plasma of patients with TAN compared with normal Nigerians. In patients, the $CNB_{12}$ as a percentage of total plasma $B_{12}$ concentration exceeded 8% in 80% of the patients, whereas it did not exceed 8% in any of the controls. Methylcobalamin was the predominant cobalamin in plasma in patients and controls, a finding similar to that reported in Caucasians (Matthews and Linnell 1971). $CoB_{12}$ was the predominant co-

TABLE 7. Plasma and liver cobalamins in patients with TAN and controls.

| | Total $B_{12}$ | Me–$B_{12}$ | CN–$B_{12}$ | $CoB_{12}$ | OH–$B_{12}$ | % CN–$B_{12}$ |
|---|---|---|---|---|---|---|
| *Liver cobalamins ($10^{-12}$ g/mg tissue)*: mean ± SE | | | | | | |
| Patients ($n = 21$) | 613 ± 150 | 4.3 ± 1.4 | 2.0 ± 0.7 | 284 ± 69 | 132 ± 46 | – |
| Controls[a] ($n = 6$) | 844 ± 280 | 11.0 ± 3.8 | 0.3 ± 0.2 | 555 ± 210 | 292 ± 84 | – |
| *Plasma cobalamins ($10^{-12}$ g/ml)*: mean ± SE | | | | | | |
| Patients ($n = 13$) | 1062 ± 199 | 525 ± 78 | 159 ± 34 | 218 ± 40 | 192 ± 60 | 13.2 ± 2.5 |
| Controls[a] ($n = 15$) | 709 ± 70 | 484 ± 62 | 26 ± 10 | 112 ± 13 | 124 ± 31 | 1.5 ± 0.6 |

[a]Controls for liver cobalamins comprised patients who died from automobile accidents or from nonhepatic, nonhematological diseases and in whom liver biopsy was obtained within 24 h of death. Members (non-smokers) of staff of U.C.H., Ibadan, served as controls for plasma cobalamins.

TABLE 8. Methylmalonic acid excretion[a] (mg/24 h: mean ± SE) in Nigerians with TAN and controls.

| | Before valine loading[b] | After valine loading[b] |
|---|---|---|
| Patients | 30.2 ± 5.0 | 31.2 ± 5.6 |
| Controls | 37.4 ± 2.9 | 34.0 ± 2.8 |

[a]Determined by the Giorgio and Plant (1965) method; Caucasians excrete in the urine < 40 mg of methylmalonic acid per 24 h (Gompertz et al. 1967).

[b]Valine was given orally at 100 mg/kg body weight (details in Osuntokun and Aladetoyinbo 1972).

balamin found in the liver in both patients and controls.

It has been suggested that because of excessive protein-binding of vitamin $B_{12}$ in Africans including Nigerians, serum $B_{12}$ concentrations may not reflect the true status of vitamin $B_{12}$ at tissue level or indicate the physiological adequacy of the vitamin. Because of the accepted role of $B_{12}$ in the detoxication of cyanide (Mushett et al. 1952; Smith and Duckett 1965) we have attempted to assess the physiological adequacy of vitamin $B_{12}$ in the Nigerian patients by measuring methylmalonic acid excretion in urine, before and after valine loading. The enzyme methylmalonyl-COA mutase, a cobamide ($CoB_{12}$) linked enzyme catalyses the isomerisation of methylmalonyl-coenzyme A to succinyl-coenzyme A. When $CoB_{12}$, the physiologically active cobalamin, is deficient, methylmalonic acid accumulates and is excreted in large amounts. Valine loading in the presence of vitamin $B_{12}$ deficiency causes an increase in methylmalonic acid excretion (Gompertz et al. 1967). Table 8 shows that methylmalonic acid excretion was normal in the Nigerian patients with TAN, and that these patients were probably not deficient in vitamin $B_{12}$ at tissue or cellular level.

## Epidemiological Studies

Nearly all the patients came from areas in Nigeria where cassava is intensely cultivated and consumed as the major or the sole dietary item.

In endemic areas for the TAN disease, there was evidence of increased exposure to cyanide in members of families where multiple or conjugal cases were found compared with members of normal families from the same endemic area (Table 9). The ataxic families consumed more cassava meals. In families in endemic areas we found significant positive correlation between age and plasma thiocyanate concentration, suggesting that prolonged exposure may be important in determining the onset of the disease (Osuntokun 1971).

The biochemical evidence of cyanide exposure in families in areas of southern Nigeria showed a remarkable trend toward equal values in members of the same family except in those who were very

TABLE 9. Metabolic studies in families in endemic areas for TAN in Nigeria.

| | No. families | No. individuals | Plasma SCN (*μmoles/100 ml*; mean ± SE) | Age (*years*; mean ± SE) |
|---|---|---|---|---|
| *Families with TAN* | 43 | 139 | | |
| Patients with TAN | | 79 | 12.7 ± 0.5 | 49.6 ± 1.5 |
| Patients with isolated opticatrophy or myelopathy | | 9 | 9.2 ± 0.8 | 41.0 ± 4.4 |
| Normals from families with neuropathy | | 55 | 6.5 ± 0.4 | 25.5 ± 2.3 |
| *Normals from normal families in endemic area* | 25 | 93 | 5.8 ± 0.2 | 37.0 ± 1.7 |
| *Normals from staff of U.C.H., Ibadan (non-smokers)* | – | 114 | 2.7 ± 0.17 | 25.0 ± 1.8 |

young (Osuntokun 1969). A common cooking pot and what is cooked appear to be more important than heredity in determining concentration of plasma thiocyanate.

Employing electrophoretic genotypes, tests for colour blindness in relatives of patients and ability to taste phenylthiocarbamide (PTC) in members of afflicted and normal families in endemic areas, there was no evidence of a genetically determined predisposition to the disease in these areas.

Results of field surveys in three villages (two in an area where cassava formed the staple diet, and the other where yam was predominantly eaten) showed that the minimum prevalence of the disease in the high cassava-eating villages was about 18–26 per thousand of the adult population (the prevalence in the 51–60-year age-group in one village was 80 per 1000) whereas the disease did not exist in the yam-eating area. We found biochemical evidence of increased exposure to cyanide in the cassava-eating areas and also some evidence that cassava farmers and processors might be more prone to the disease (Osuntokun et al. 1969a, b; Osuntokun 1969, 1972).

## Neuropathology

So far in our experience, no patient has died from the disease, so that postmortem studies have not been possible.

Predominantly posterior column myelopathy was the commonest clinical feature closely followed by optic atrophy and perceptive deafness. The peripheral nerves appear to be the last to be involved. Studies of peripheral nerves obtained at biopsy have shown segmental demyelination both on electronmicroscopy and by single nerve fibre preparations (Williams and Osuntokun 1969; Osuntokun 1970a). Nerve conduction velocity was reduced, sometimes even in those without clinical evidence of neuropathy. The evidence available suggests that chronic cyanide intoxication can induce demyelination in experimental animals by selective and primary effects on glial cells especially oligodendroglia (Bass 1968) and also perhaps on Schwann cells (Williams and Osuntokun 1969) and may also cause neuronal necrosis. High thiocyanate levels in the Nigerian patients may also predispose to chronic cyanide intoxications, since thiocyanate can be converted to cyanide by thiocyanate oxidase in haemoglobin (Chung and Wood 1971).

We have shown that cassava diet fed to Wistar rats for 18–24 months caused raised plasma thiocyanate levels and that these rats develop ataxic and segmental demyelination (Osuntokun 1969, 1970b; Osuntokun and Williams unpublished data).

The pattern of loss of visual field in most of the patients supports the hypothesis of a diffusible toxin such as cyanide which attacks the relatively unprotected peripheral retinal receptors and the macula. This accounts for the high frequencies of concentric diminution of visual fields and temporal pallor in patients in whom marked optic atrophy had not developed (Osuntokun and Osuntokun 1971). Presumably cyanide, released from cyanocobalamin ("the Trojan horse"—Lancet 1969) by photolysis or the effect of electromagnetic waves in the visual spectrum, may then accumulate in the vitreous and damage the retinal receptors. Damage to the papillomacular bundle may explain the frequency of temporal pallor. Progressive and continuous damage leads to complete optic atrophy. A combination of damage to papillomacular bundle and the peripheral retinal receptors may be expected to occur. Changes in optic nerves have been described in cyanide poisoning in primates (Ferraro 1933; Hurst 1940). Cyanide in sublethal doses for up to 3 weeks in rats caused demyelination in optic nerves and retina in addition to callosal lesions (Lessell 1971). I have already referred to the frequency of dementia in patients (9%) with tropical ataxic neuropathy (Osuntokun 1969). Could this be a result of callosal demyelination?

The nature of the perceptive deafness which indicates both receptor organ and neuronal lesions (Osuntokun et al. 1970c, Hinchcliffe et al. 1972) is compatible with the reported effects of cyanide on the chick-embryo otocyst (Friedmann 1972).

## Goitre and Cyanide Intoxication

The prevalence (2.5%) of goitre in Nigerian patients with TAN is higher than in the general population and may be related to cassava diet (Osuntokun 1971). There is also a distinct possibility that the southern belt in Western Nigeria where the incidence of goitre is high (Oluwasanni and Alli 1968) is due to high consumption of cassava. Thiocyanate, a detoxication product of cyanide, is a well-known goitrogenic substance.

TABLE 10. Urinary and serum riboflavin and serum caeruloplasmin[a] in Nigerian patients with TAN.

| | Riboflavin | | Serum caeruloplasmin (*mg/ml*; mean ± SE) |
|---|---|---|---|
| | Urinary (*μg/g creatinine*; mean ± SE) | Serum (*$10^{-9}$ g/ml*; mean ± SE) | |
| Patients with TAN | 51.5 ± 3.9 ($n = 54$) | 19.34 ± 0.9 ($n = 30$) | 1.17 ± 0.07 ($n = 44$) |
| Normals from endemic area | 48.4 ± 2.8 ($n = 46$) | 18.8 ± 0.6 ($n = 34$) | – |
| Non-neurological patients (U.C.H., Ibadan) | 81.0 ± 2.5 ($n = 25$) | 25.4 ± 1.3 ($n = 36$) | – |
| Controls (staff of U.C.H., Ibadan) | 84.0 ± 2.6 ($n = 36$) | 28.2 ± 0.8 ($n = 163$) | 1.44 ± 0.07 ($n = 50$) |

[a]Urinary riboflavin was measured according to the ICNND Manual method (1963), serum riboflavin according to Burch et al. (1949) using a model 27 direct reading fluorimeter (Electronics Instruments Ltd., Richmond, England), and serum caeruloplasmin by the quantitative radial immunodiffusion technique employing the antibody incorporated agar method of Mancini et al. (1965) (the immunoplates were obtained from Hyland Laboratories, Los Angeles, Calif., U.S.A.).

Ekpechi (1967) demonstrated that cassava is goitrogenic in experimental animals.

## Other Investigations

Results of the biochemical, serological, electrophysiological, and histological investigations have been presented in detail elsewhere (Osuntokun 1968, 1969, 1970a; Osuntokun et al. 1968; Williams and Osuntokun 1969; Osuntokun and Williams, 1970; Aladetoyinbo et al. 1971). There was no biochemical evidence of protein-calorie deficiency. Serum transferrin said to be a sensitive index of protein nutritional status was normal. Red blook cell transketolase activity, and excretion in urine of thiamine and N-methylnicotinamide and serum folate, were normal. Concentrations of serum calcium, phosphate, sodium, potassium, bicarbonate, and tests of thyroid, hepatic, and renal functions were normal. Amino acids and porphobilinogen were absent in urine. Occasionally, schistosoma ova were found in urine. There was no biochemical evidence of malabsorption. Glucose tolerance tests with and without stressing by prednisolone were not significantly abnormal. Histamine-fast achlorhydria was rare. Serologic tests for syphilis, typhoid, typhus, brucellosis, and screening for prevalent viral infections gave usually negative results. There was no increased prevalence of malaria and urinary tract infections were not encountered in patients. Electrocardiograph examination showed no significant abnormalities.

## Deficiency of Riboflavin and Caeruloplasmin

However, we found diminished urinary excretion of riboflavin and low serum riboflavin and caeruloplasmin levels in patients compared with controls (Table 10).

The biochemical effects of deficiency of riboflavin may combine with those of cyanide intoxication. However the mucocutaneous lesions classically attributed to ariboflavinosis, present in 35% of patients with TAN, are now regarded as nonspecific evidence of diminished cellular oxidation in rapidly regenerating tissues such as the skin and mucous membranes. They may therefore be present in other deficiency states involving iron, thiamine, vitamin $B_{12}$, nicotinic acid, and cyanide-induced inhibition of cytochrome oxidase. Retrobulbar neuritis may result from riboflavin deficiency, but deafness and myelopathy have so far not been reported (Goldsmith 1964). Riboflavin-responsive normocytic, normochronic, or micro-

cytic hypochronic anaemia with vacuolation of the erythroid and myeloid cells of the bone marrow reported in experimental riboflavin deficiency, in kwashiorkor, and in other deficiency states in man such as alcoholism, phenylalanine deficiency, and in association with chloramphenicol administration, has never been encountered in TAN. Anaemia was absent and marrow was normal in patients. Riboflavin deficiency is common in most southern Nigerians (Edozien 1965; U.S. Report 1967) probably due to repeated and prolonged cooking of vegetables, which destroys the vitamin. It is unlikely that riboflavin deficiency plays a major role in the etiology of TAN.

Deficiency of caeruloplasmin as in Wilson's disease may cause neuropathological lesions. Although serum caeruloplasmin level in patients was lower than in normal Nigerians, TAN is clinically unlike Kinnier Wilson's disease. Histology of liver biopsy specimens from the Nigerian patients showed no evidence of cirrhosis or copper deposition and aminoaciduria was absent (Osuntokun 1968, 1969). An interesting speculation is that deficiency of caeruloplasmin may be cyanide-induced as cyanide is known to reversibly inhibit the synthesis or the oxidase activity of caeruloplasmin (Speyer and Curzon 1968). It may be worthwhile to estimate tissue and plasma copper in the Nigerian patients, because of the relationship of copper deficiency to "sway-back" in sheep, a disease characterised by ataxia, blindness, and demyelination of the cerebral hemispheres and spinal cord (Innes and Saunders 1962).

## Therapeutic Trials

On the hypothesis that chronic cyanide intoxication and riboflavin deficiency are the major etiological factors, we conducted two double-blind controlled trials in TAN. In the first trial we administered to three randomised groups of patients weekly intramuscular injections of hydroxocobalamin (1000 $\mu$g), riboflavin (5 mg), and placebos which were given for 24 weeks. In the second trial, in three randomised groups, weekly injections of hydroxocobalamin (5000 $\mu$g) plus cystine tablets (500 mg) daily, weekly injections of 5 mg of riboflavin plus cystine tablets (500 mg) daily were compared with placebos and given for 48 weeks. No beneficial effects were demonstrable by objective assessment (Osuntokun et al. 1970, 1973 in press). This could, of course, be due to the chronicity of the nervous lesions. We are now studying the effects of administration of hydroxocobalamin, riboflavin, cysteine, and cystine on urinary excretion of thiocyanate and prevention of neuropathy in Wistar rats fed a cassava diet.

## Conclusion

I believe that the evidence available so far incriminates chronic cyanide intoxication of dietary origin as the major etiological factor in TAN in Nigerians and also perhaps in Tanzanians (Makene and Wilson 1972). The main source of the cyanide in Nigerians is the culinary derivatives of cassava. Why many Nigerians who eat cassava meals do not develop the disease may be due to variation in biological susceptibility, the way the cassava is prepared and eaten, the species of cassava, and the type of other food items and supplements (especially the amount of animal protein). For example not all prisoners of war subjected to nutritional deprivation in the Far East during World War II developed symptoms of deficiency disease (Victor and Dreyfus 1961). Besides, the concentration of cyanogenic glycosides in cassava root depends on the species, season, humidity, type of soil on which it is grown, amount of rainfall, and time of harvesting. For example, cassava contains more hydrocyanic acid in December than in September (Oyenuga and Amazigo 1957). The hydrocyanic acid released on hydrolysis of cassava derivatives depends on the way these derivatives are prepared. Concomitant large intake of animal protein may protect against the harmful effects of a cassava diet. TAN is a disease of poor people, and in Nigeria has never been encountered among those in the high socio-economic groups.

Although there are similarities between TAN in Nigerians, and some "nutritional" neuropathies seen in other tropical and subtropical areas (for review see Osuntokun, 1968, 1969, 1971) it is not justifiable to assume that these represent clinical variants of the same disease and are etiologically related to chronic cyanide intoxication. When a diet is poor, multiple nutritional deficiencies usually occur together. While one single factor may be most important, other factors may coexist and combine to produce the final picture. In Nigerian TAN, cassava diet causing chronic cyanide intoxi-

cation appears to be the most important factor in the onset of the disease. Preventive measures such as cultivation of cassava species and production of cassava derivatives low in cyanogenic glycosides and improved socio-economic standards are likely to be the most effective methods of reducing the prevalence of the disease, which in some areas of Nigeria is as high as 3%.

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# Endemic Goitre and High Cassava Diets in Eastern Nigeria

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**Abstract** Subsequent to the discovery of patchy distribution of endemic goitre and the low but markedly varying environmental iodine deficiency in eastern Nigeria, experimental studies of the possible goitrogenic action of dry, unfermented cassava on rats were undertaken. The results showed that cassava has an adverse action on the function of the thyroid, an action comparable to that of thionamide goitrogen.

**Résumé** La répartition inégale du goitre endémique et la déficience environnementale, faible mais notablement variable, de l'iode dans le Nigéria oriental, nous ont incité à entreprendre une étude expérimentale, sur des rats, de l'action du manioc séché non fermenté comme agent causatif possible du goitre. Les résultats démontrent que le manioc a une action adverse sur la fonction thyroïdienne, action comparable à celle de la thionamide.

AN endemic goitre survey (Ekpechi 1964) in Nsukka Division of the east central state of Nigeria in Africa disclosed a marked variation in incidence of goitre from village to village. Also there was no consistent inverse relationship between the iodine content of the drinking water from the various villages and the incidence of goitre. Eha-Amufu village has a visible goitre incidence of 38.2% and a drinking water iodine content of $2.75 \pm 0.04$ μg/litre compared to Nsukka village with a much lower visible goitre incidence of 9.3% and drinking water iodine content of $0.86 \pm 0.09$ μg/litre. A crude dietary survey suggested a correlation between the intake of dry, smoked, unfermented cassava and the incidence of goitre. Animal experiments designed to test the effect of dry, cooked, unfermented cassava on thyroid function were subsequently carried out at the Royal Postgraduate Medical School of London University and Hammersmith Hospital, London, in Professor T. Russel Fraser's Laboratory.

## Preparation of Cassava

The preparation of cassava for food varies. In most areas the cassava is fermented by soaking in water for 3 days, then processed to yield a white, wet, soft, smooth, tasteless paste. The processed product is boiled in water at 160°C for 30 min, pounded, then boiled again for 30 min before pounding to a rubbery paste. It is then ready to be swallowed with gravy.

Other methods of preparation require boiling or frying after the initial fermentation. The finished product of fermented cassava is white or greyish white. Preparation of dry and unfermented cassava consumed in the areas of Nsukka Division is different. The raw cassava is peeled, smoked, then

pulverized and mixed with water to form a greyish-white paste which is then smoked for many months. To prepare a meal of unfermented cassava, the greyish white dried cake is again pulverized and added to boiling water and stirred until a bluish-black paste is formed. This is then cooled and served with gravy.

## Materials and Methods

Young female albino rats (Sprague Dawley strain) weighing 170–190 g were divided into four groups of three rats each. The first group was fed 100% cassava diet (prepared in the traditional way), the second group 50% cassava diet mixed with 50% standard laboratory diet (41B cubes), the third group 100% cassava diet plus iodine supplement (10 $\mu$g/rat per day), and the fourth group standard laboratory diet. The diets were given ad libitum for 7 days. The rats were injected intraperitoneally with 20 $\mu$c $^{131}$I 24 h before being killed, subsequently anaesthetised with ether, and exsanguinated. The thyroids were quickly removed, counted for radioactivity, and enzymatically digested. The digest was purified by passing through a 6-inch column of anionic exchange resin (Dowex 1 × 2, 200–400 mesh) and chromatographed in an ascending butanol–acetic acid–water solvent system. Estimates of the stable iodine content of the thyroids were made on aliquots of the digests using the alkaline ashing method.

## Results

The rats on cassava diet alone appeared to lose appetite about the fourth day of the experiment and their general food intake was less than that of rats on either standard laboratory diet (41B cubes) or those on iodine supplement: 16 g/rat per day compared to 20 g/rat per day. The animals on iodine supplement were very active, scattering their food all over their cages and defecating and urinating more than the other animals.

### Thyroid Weight

The mean thyroid weight of rats on standard laboratory diet was $7.3 \pm 0.06$ mg compared to $11.1 \pm 1.1$ mg for rats on 100% cassava, 9.1 mg for rats on 100% cassava plus iodine supplement, and $10.2 \pm 1.4$ mg for rats on 50% cassava.

The mean thyroid weight of rats on either 100 or 50% cassava seems to be higher than for the other groups, and even with these small groups, the thyroid weight of those on 100% cassava was probably significantly higher than the controls ($t = 2.94$, $P < .05$).

### Thyroidal $^{131}$I Uptake at 24 h

The mean thyroidal $^{131}$I uptake of the rats on standard laboratory diet was $14.4 \pm 0.7$% dose, compared to 18.2% for rats on 100% cassava and $14.3 \pm 0.7$% dose for rats on 50% cassava.

The animals fed 100% cassava had a probably significantly higher uptake than the controls ($t = 5.5$, $P < .01$) whereas the other three groups differed very little (those on 50% cassava appeared to have a lower uptake; $t = 3.2$, $P < .05$). This higher uptake was no longer present in animals fed 100% cassava plus iodine supplement, whose uptake was normal.

### Serum Protein-Bound Radioactive Iodine

The following 24-h protein-bound $^{131}$I values are expressed as percent dose per cubic centimeter of serum: 0.028 for rats fed standard laboratory diet compared to 0.08 for those fed 100% cassava, 0.06 for those fed 100% cassava plus iodine supplement, and 0.02 for those fed 50% cassava.

The animals on 100% cassava had a PB $^{131}$I 2.9 times that for rats on control diet. Note that the level of PB $^{131}$I remained still higher in animals fed 100% cassava plus iodine. Only single estimations on the pooled sera of the different groups of rats could be done as the serum from one rat was not found sufficient for a valid estimation. Statistical testing of the difference in PB $^{131}$I value between the different groups could not be done. I previously reported (1964) a mean PB $^{131}$I of $0.017 \pm 0.006$% dose/cm$^3$ for 15 rats on standard laboratory diet and comparing this (probably) normal value with the value of 0.08 found for rats on 100% cassava or 0.06 for rats on 100% cassava plus supplement suggests that the PB $^{131}$I for 100% cassava-fed rats was still markedly elevated.

### Thyroidal $^{127}$I

*Total concentration*—The value expressed in micrograms per milligrams thyroid tissue for rats on standard laboratory diet was 0.8 compared to 0.14 for rats on 100% cassava, 0.36 for rats on

100% cassava plus iodine supplement, and 0.35 for rats on 50% cassava. As these estimates were done on the pooled homogenate of each group of three rats it was not possible to test statistical significance of the difference between the different groups, but with a coefficient of variation of 12% as found for similar estimates on 15 rats on standard laboratory diet, it is quite evident that there is a striking difference between the values of 0.14 μg/mg for rats on 100% cassava and 0.8 μg/mg for rats on standard laboratory diet.

*Total stable*—The mean total stable iodine for rats on standard laboratory diet was 10 μg/gland or 5.9 μg/100 g body weight compared with 2.6 μg/gland or 1.6 μg/100 g body weight for those on 100% cassava, 6.7 μg/gland or 3.6 μg/100 g body weight for rats on 50% cassava, and 5.8 μg/gland or 3.3 μg/100 g body weight for those on 100% cassava plus iodine supplement. This degree of depletion of iodine stores in rats fed 100% cassava is quite noteworthy.

### Chromatographic Studies

As shown in Fig. 1, the animals on standard laboratory diet had an MIT : DIT ratio of $0.56 \pm 0.04$ compared to $1.44 \pm 0.06$ for rats on 100% cassava, $1.3 \pm 0.02$ for those on 100% cassava plus iodine supplement, and $1.4 \pm 0.1$ for those on 50% cassava. The MIT : DIT ratio for rats either on 100% cassava ($t = 14.6$, $P < .001$) or on 100% cassava plus iodine ($t = 12.8$, $P < .001$) is significantly higher than that for rats on standard laboratory diet.

The proportion of $^{131}I$ as iodothyronine (i.e. thyroxine and triiodothyronine), 35.6% of the organic for rats on standard laboratory diet, is much lower than that for the other three groups of animals. There is a high peak of activity beyond the site of thyroxine as identified with stable thyroxine (Fig. 1, top). It is likely, but not definite, that this peak corresponds to the site of triiodothyronine in this solvent system (butanol–acetic acid).

The proportion of $^{131}I$ as iodothyronine for rats on 100% cassava was 51% compared to 64% for rats on 100% cassava plus iodine supplement and 50% for rats on 50% cassava.

## Discussion

There is recent experimental support that some antithyroid agents, such as thiourea, have a variable effect on the radioactive iodine uptake (Slingerland et al. 1959). They reported that propylthiouracil at a concentration of $1 \times 10^{-4}$ mg irregularly stimulates radioactive iodine uptake in the rat thyroid, while at higher concentration it regularly depresses uptake. Kilpatrick (1961) also reported that rats fed 100 μg of carbimazole daily for 10 days had a radioactive iodine uptake which was significantly higher than that for rats on water or 500 μg of carbimazole daily for 10 days. The figures for the 24-h uptakes were 10.1% dose for control rats, 6% dose for those on 500 μg of carbimazole, and 13.5% dose for those fed 100 μg of carbimazole.

These observations raise the possibility that small daily intakes of dietary goitrogens in endemic areas could cause an increase rather than a decrease in radioactive iodine uptake. Clement and Wishart (1956) reported that milk containing a suspected goitrogen depressed radioactive iodine uptake in adults who drank it. But the amount of milk consumed (60–80 oz) was certainly a much larger quantity than a normal adult takes daily. The larger the amount given, the greater the amount of probable goitrogen ingested, and the pattern of uptake may well change. It would be interesting to see what would happen to the uptake if Clement and Wishart had given only 10–20 oz of milk to their patients.

The probably significantly higher thyroid weight and higher thyroidal uptake of radioactive iodine at 24 h for rats on 100% cassava, compared with rats on standard laboratory diet, suggest an effect of iodine deficiency on the thyroid. But a pure iodine-deficient diet did not significantly change the thyroid weight even after 206 days so this effect of cassava on thyroid uptake is unlikely due to its low content of iodine alone. This observation, that pure iodine deficiency does not cause an early change in thyroid weight, has been made by van Middlesworth (1952) and Money et al. (1952).

More recent work by Slingerland et al. (1959) and Kilpatrick (1961) has shown that low concentration of a goitrogen of the thionamide series can cause an increase or variable thyroidal uptake of radioactive iodine. It is, therefore, possible that the increased uptake of $^{131}I$ in cassava-fed animals (100%) could have been due to the presence of a low concentration of a goitrogen in cassava.

The striking degree of thyroidal iodine depletion caused by cassava in 7 days is unlikely to have been

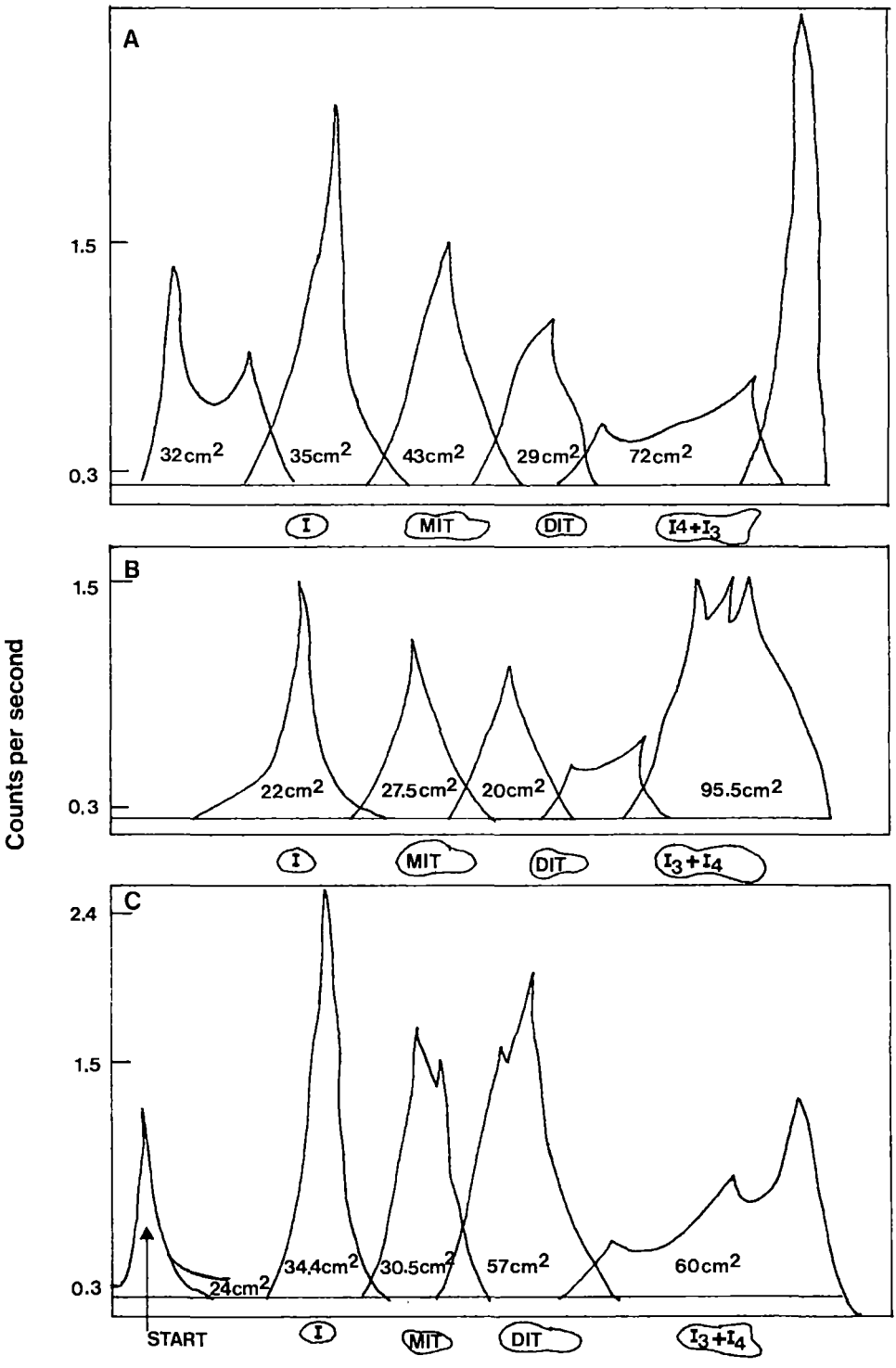


FIG. 1. Effect of cassava on thyroid hormone production in the rat (chromatograms of resin purified rat thyroid digests after 7 days on cassava).

TABLE 1. Comparison of various measures of thyroid function in rats fed on 100% cassava with similar measures on rats on 100% cassava plus iodine supplement, 50% cassava, and standard control diets over a 7-day period.

| Experimental groups | Thyroid weight (*mg/100 g/rat*) | Thyroid digest: Distribution of Intrathyroidal $^{131}I$ at 24 h as % dose: Ratio MIT:DIT (mean ± SE) | Thyroid digest: Distribution of Intrathyroidal $^{131}I$ at 24 h as % dose: Iodothyronine as % organic (mean ± SE)[c] | Total $^{127}I$[a] (*μg/mg thyroid*) | S PB $^{131}I$ % dose/cc |
|---|---|---|---|---|---|
| Fed 100% Cassava | 11.1 ± 1.1 | 1.44 ± 0.06[c] | 51% | 0.14 | 0.08 |
| 50% Cassava plus 50% Control Diet (41B) | 10.2 ± 1.4 | 1.4 ± 0.11[c] | 50% | 0.35 | 0.02 |
| Cassava plus iodine supplement (*μg/rat/day*) | 9.1 ± 1 | 1.3 ± 0.02[b] | 65% | 0.36 | 0.06 |
| Control diet 41B and standard laboratory diet | 7.3 ± 0.06 | 0.6 ± 0.02 | 35.6% | 0.8 | 0.028 |

[a]Single estimates on pooled homogenate of three rats.
[b]Single estimates on pooled sera of three rats.
[c]Iodothyronines as % of organic iodoamino acids.

caused by its low iodine content. Table 1 shows that a 7-day pure iodine-deficient diet had no obvious effect on the total thyroidal iodine. Slingerland et al. (1959) and Kilpatrick (1961) reported severe depletion of total thyroidal iodine by thionamide goitrogen. Slingerland et al. (1959) found that the total thyroidal iodine of the rat was reduced from 9 μg/gland to 2.4 μg/gland in 10 days by 0.1% propylthiouracil. Kilpatrick (1961) found that carbimazole (500 μg) reduced the total thyroidal iodine in the rat from 15.2 to 3.6 μg in 10 days. In this respect the effect of cassava on the total iodine stores is not unlike that of either carbimazole reported by Kilpatrick or to 0.1% propylthiouracil by Slingerland et al.

The marked elevated PB $^{131}I$ as found in 100% cassava-fed rats could be regarded as a reflection of the low intrathyroidal iodine stores and the rapid turnover. But this degree of elevation of PB $^{131}I$ was not observed with pure iodine deficiency. Such increased release of thyroidal $^{131}I$ either as reflected by the PB $^{131}I$ or otherwise has been observed in rats fed propylthiouracil by van Middlesworth (1952) and by Escobar del Rey et al. (1961). Using $^{131}I$ labelled l-thyroxine, they later reported that such increased release of thyroidal $^{131}I$ in rats treated with propylthiouracil and thiouracil was due to the decreased peripheral deiodination of thyroxine. Such effects of thionamide goitrogens (propylthiouracil and thiouracil) in decreasing peripheral deiodination of thyroxine can and would cause a marked elevation of PB $^{131}I$. This abnormal elevation of PB $^{131}I$ in 100% cassava-fed rats is, therefore, possibly due to the presence of a thionamide goitrogen in cassava, since as mentioned above, very low intrathyroidal iodine concentration alone did not produce that degree of elevation in our other experiments.

The significant changes in the intrathyroidal iodine metabolism are the alteration of ratio of $^{131}I$ MIT:DIT and the increased production of iodothyronines in all cassava-fed animals compared to the rats on standard laboratory diet. It is worthy of note that even iodine supplementation did not reverse this effect of cassava on thyroid function.

Changes in the ratio of $^{131}I$ MIT:DIT have been reported in a number of conditions, and it is doubtful if this change is specific to any pathological or biochemical abnormality in the thyroid. Nevertheless, this change has consistently been reported in animals fed thionamide goitrogens. Kilpatrick (1961) reported that the ratio of $^{131}I$ MIT:DIT for rats on 100 μg of carbimazole for 10 days was

1:34. Slingerland et al. (1959) found a ratio of 2:2 for rats on 0.1% propylthiouracil for 10 days. No one as far as we know has reported a change in this ratio in the very early stages of iodine deficiency. Leloup and Lachiver (1955) reported changes in this ratio in rats after 3 or 6 months on an iodine-deficient diet. We found a normal ratio of 0.34 in albino rats fed an iodine-deficient diet after 7 days.

Slingerland et al. (1959), discussing the change in this ratio, commented that a severe depletion of total iodine in the gland was not the cause since the MIT:DIT remained high when the total iodine was preserved by adding thyroid to propylthiouracil. A high T.S.H. as well could not account for the elevated ratio since thyroid tablets would have suppressed this. Ermans et al. (1963) found a significant correlation ($r = 0.73$, 0.02, $P < 0.01$) between this ratio of MIT:DIT and iodine concentration per gram, but not with total iodine of human goitrous thyroid gland in "slow secretion patients" from Uele in the Congo, an endemic goitre area. On the other hand, Dimitriadou et al. (1961) did not find any change in this ratio in young Thai endemic goitres even though the iodine concentration per gram of thyroid in some patients was as low as that of Ermans et al. (1963).

After 7 months on an iodine-deficient diet the MIT:DIT ratio of rats was still below unity regardless of the fact that their iodine concentration per milligram of thyroid tissue had dropped to 0.14 $\mu$g. In the light of our experimental results as stated above, the change in the $^{131}$I-labelled MIT:DIT found in cassava-fed animals must have an explanation other than diminished iodine concentration or diminished total iodine. And the most likely explanation here is that cassava has an effect on this ratio not unlike that of the thionamide goitrogens.

The increased proportion of $^{131}$I as iodothyronines in all cassava-fed animals compared to rats on standard laboratory diet is a reflection of the rapid iodine turnover which in turn reflects the decreased thyroidal iodine concentration in these animals. Such an accelerated release of labelled hormone and rapid turnover in the pattern in human thyrotoxicosis and iodine deficiency has been reported by Dimitriadou et al. (1961) and by Kierderling et al. (1961). So far as we are aware such over-active synthetic function of the thyroid has not been reported with goitrogens. On the contrary, it is well established that goitrogens depress production of iodothyronines. Studies which recorded the depression of iodothyronine production by thionamide goitrogen have been carried out with chemically pure thionamide goitrogen and the general conclusions from such studies can not apply strictly to our studies since the cassava effect on thyroid function is a combination of its iodine deficiency as well as its probable goitrogenic content. This unusual combined effect of high MIT:DIT ratio and increased rate of production of iodothyronine in rats fed 100% cassava can probably be explained by the very low thyroidal iodine concentration and the rapid turnover of whatever iodine that gets beyond the block between the MIT and DIT.

As can also be observed in Table 1, the rate of iodothyronine production is higher (64%) in animals fed cassava plus iodine supplement than in animals on cassava alone (51%). It seems that there are two factors responsible for this observation: the increased rate of secretion of labelled active hormone as reflected by PB $^{131}$I, and the probable increased rate of production of triiodothyronine in animals on cassava alone without iodine supplement (0.08% dose against 0.06% dose).

The above observations of the effect of unfermented cassava on different parameters of thyroid function strongly suggest that unfermented cassava contains a substance with an effect not unlike that of the thionamide goitrogens. Thus, the probably increased thyroid weight, the abnormally elevated PB $^{131}$I, the severe depletion of iodine stores and the higher ratio of MIT:DIT, and also the fact that iodine supplement could not reverse all the effects of cassava, are in keeping with the effect of a thionamide goitrogen.

The point has been made that food goitrogens act as permissive factors in an area of poor iodine supply. Some support to the above comes from our therapeutic trials of pot iodide in an unselected group of goitrous patients in Nsukka Division. Among 236 young goitrous adults living in this division, aged 25 and under, given 10 mg of pot iodate daily for 3 months, only 17 still had palpable glands after the trial whereas the response to iodine therapy was very poor in the older age-group from the same village. The trial was confined to 67 non-nodular goitrous males and females aged 45 years and over. Only 11 had any marked decrease in their goitre size after 3 months. A longer duration of iodine therapy may be needed

to produce significant reduction in the size of older goitres.

When sufficient research funds are available for the detailed study of iodine kinetics, and the measurement of thyroidal uptake of $^{131}I$ before and after cassava meals, we will be able to determine what role dry, smoked, unfermented cassava plays on the etiology of goitre in the inhabitants of this area.

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# Evidence of an Antithyroid Action of Cassava in Man and in Animals[1,2]

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DELANGE, F., M. VAN DER VELDEN, AND A. M. ERMANS. 1973. Evidence of an antithyroid action of cassava in man and in animals, p. 147–151. *In* Chronic cassava toxicity: proceedings of an interdisciplinary workshop, London, England, 29–30 January 1973. Int. Develop. Res. Centre Monogr. IDRC-010e.

**Abstract** Previous epidemiological and metabolic studies concerning the etiology of endemic goitre on Idjwi Island (Kivu Lake, Republic of Zaïre) led to the conclusion that iodine deficiency was not the single causal factor. Indeed, goitre prevalence exhibited striking regional variations although the whole island was subjected to a severe and uniform iodine deficiency, and the possible role of a dietary goitrogen was suspected. The present work was undertaken in order to detect an antithyroid activity, in men and in rats, of the foods eaten in this area.

The absorption of cassava in men induces an inhibition of the penetration of iodide into the thyroid as expressed by a considerable drop in radioiodine thyroidal uptake and a rise in urinary excretion of stable and labelled iodide. Inhibition of thyroid uptake is also obtained in rats fed with cassava and this effect is accompanied by a striking rise in the plasma thiocyanate concentration and urinary thiocyanate excretion. These results are similar to those obtained in separate experiments where rats were fed thiocyanate.

These investigations show that cassava grown on Idjwi Island, an endemic goitre area, has an antithyroid action in men and in rats. Cassava could constitute a dietary goitrogen responsible, at least partially, for endemic goitre in this area. The antithyroid action of cassava is due to thiocyanate. Thiocyanate is probably endogenously produced from cyanide, which is released by a cyanogenic glucoside present in large quantities in cassava.

**Résumé** Des études épidémiologiques et métaboliques antérieures sur l'étiologie du goitre endémique dans l'île d'Idjwi (lac Kivu, République de Zaïre) ont démontré qu'une carence d'iode n'est pas la seule cause de cette maladie. En fait, la fréquence du goitre accuse des variations régionales remarquables, bien que l'île entière souffre d'une carence d'iode sérieuse et uniforme. On soupçonne la présence possible d'un agent causatif du goitre dans le régime alimentaire. La présente étude a été entreprise dans le but de déceler, chez l'homme et le rat, une activité antithyroïdienne attribuable aux aliments mangés dans la région.

L'absorption du manioc chez l'homme empêche l'iode de pénétrer dans la thyroïde, comme l'indiquent une baisse considérable de la captation d'iode radioactif par la thyroïde et une augmentation d'iode stable et marquée dans l'urine. Un régime à base de manioc empêche la captation

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d'iode par la thyroïde chez le rat également. Cet effet est accompagné d'une élévation notable de la teneur en thiocyanate du plasma et de l'élimination de thiocyanate dans l'urine. Ces résultats sont en accord avec ceux d'essais séparés au cours desquels des rats ont été nourris de thiocyanate.

Ces recherches démontrent que le manioc cultivé dans l'île d'Idjwi, région où le goitre est endémique, a une action antithyroïdienne chez l'homme et chez le rat. Il est donc possible que le manioc soit l'aliment responsable, du moins en partie, du goitre endémique dans la région. L'action antithyroïdienne du manioc est due au thiocyanate. Le thiocyanate est probablement produit par réaction endogène à partir du cyanure, qui est lui-même libéré par un glucoside cyanogène présent en grande quantité dans le manioc.

THE role played by cassava in the etiology of endemic goiter has been suspected by several researchers on the basis of observations made in Nigeria (Nwokolo et al. 1961; Ekpechi 1967; Oluwasanmi and Alli 1968; Osuntokun 1971). This hypothesis has been supported by our investigations carried out in Idjwi Island, Kivu Lake, Republic of Zaïre. An epidemiological survey covering the entire population of the island showed very important regional variations of goiter prevalence, which reached 55% in the north of the island and only 5% in the south (Delange et al. 1968). The severity of this endemia is emphasized by the endemic cretinism prevalence, which reaches 1.1% of the population. Several metabolic studies showed that a difference of iodine deficiency cannot account for the epidemiological differences observed between the north and south. Indeed, an extremely severe iodine deficiency has been observed in the goitrous area as well as in the nongoitrous area (Thilly et al. 1972). These observations, and the fact that in other parts of the world subjected to severe iodine deficiency no endemic goiter has been observed (Roche 1959; Choufoer et al. 1963; Riviere et al. 1968), led us to the hypothesis that iodine deficiency is only a permissive factor in the etiology of endemic goiter in man. To explain the epidemiological observations made in Idjwi, we systematically surveyed the differences between the environmental factors in the goitrous and nongoitrous areas of the island. A nutritional survey did not reveal quantitative or qualitative differences between the two regions, except for cassava, the consumption of which is higher in the goitrous area than in the nongoitrous area. Furthermore, the nature of the soil is different in the two regions; in the north, granite, and in the south, basalt (Ermans et al. 1969).

Table 1 shows that the plasma thiocyanate (SCN) concentration observed in the inhabitants of Idjwi is four times higher than in the Belgian controls and that urinary excretion of SCN is higher in the goitrous area than in the nongoitrous one ($P < 0.001$). The presence of abnormal amounts of SCN in blood and urine may be considered evidence of the presence of a goitrogenic factor in the foodstuff (Silink 1964).

TABLE 1. Thiocyanate levels in plasma and urine in Idjwi inhabitants and Belgian controls (number of samples shown in parentheses; data from Ermans et al. 1969).

| | Plasma SCN concn (*mg/100 ml* ± SE) | Renal SCN excretion (*mg/24 h* ± SE) |
|---|---|---|
| Goitrous area | 1.10 ± 0.09 (56) | 14.3 ± 1.5 (30) |
| Nongoitrous area | 1.07 ± 0.11 (56) | 10.0 ± 0.9 (47) |
| Belgian controls | 0.24 ± 0.03 (30) | – |

## Metabolic Studies in Man

To detect the antithyroid action of foods consumed in Idjwi, we compared the ingestion of several foods on the thyroidal uptake of radioiodine (Delange et al. 1971). The above-mentioned observations led us to focus our investigation on cassava (*Manihot* spp.). The foods considered were cassava, bananas, peanuts, and pumpkins. They were provided from fields located near the dwellings of the subjects studied and were prepared in the customary manner. One group of subjects was fed rice imported from Europe, used as a goitrogen-free control food. Ten microcuries of $^{131}I$ were administered orally immediately after ingestion of the meal and thyroidal uptake of $^{131}I$ was estimated 24 h later. Table 2 shows the results of this investigation. The mean values of thyroidal uptake observed in patients who had eaten bananas, peanuts, and pumpkins are comparable to those of the control group. By contrast, the mean values in patients who had eaten cassava are significantly lower. Additional investigations showed that this effect is accompanied by an increase in

TABLE 2. Influence of the ingestion of different foods on thyroid uptake of radioiodine in the goitrous and nongoitrous areas of Idjwi Island (data from Delange and Ermans 1971).

| Foods | No. subjects | Avg amount ingested (*g*) | $^{131}I$ thyroid uptake (*24 h, % dose*) | *t*-test (*P*)[a] |
|---|---|---|---|---|
| *Goitrous area* | | | | |
| Rice (control group) | 22 | 315 | $86.8 \pm 2.4$ | |
| Cassava | 27 | 490 | $71.9 \pm 2.4$ | $<0.001$ |
| Bananas | 10 | 675 | $85.4 \pm 3.7$ | $>0.5$ |
| Peanuts | 10 | 165 | $92.5 \pm 1.7$ | $>0.1$ |
| Pumpkins | 10 | 520 | $79.5 \pm 3.5$ | $>0.05$ |
| *Nongoitrous area* | | | | |
| Rice (control group) | 22 | 505 | $73.5 \pm 2.1$ | |
| Cassava | 10 | 475 | $74.8 \pm 2.1$ | $>0.5$ |

[a]Compared with the control group (rice).

urinary excretion of labelled iodine. Similar results are shown in Fig. 1, which concerns an investigation performed on one of the authors (F.D.), and shows the comparison of curves of thyroidal uptake of radioiodine obtained in the same subject after a control meal and after ingestion of a meal of cassava grown in the goitrous area of the island. Urine was completely collected over 24 h following the administration of the tracer. The curve obtained after ingestion of cassava shows a clear slowdown of the thyroidal uptake of $^{131}I$ compared with the curve obtained after a rice meal. This change is accompanied by an increase in urinary excretion of radioiodine per day, the reduction in thyroid trapping corresponding quantitatively to an increase in its excretion in the urine. This effect is associated with a sharp increase in urinary excretion of stable iodine. These studies show that the absorption of cassava grown in the goitrous area of Idjwi brings about partial inhibition of iodine uptake by the thyroid and an increase of its renal excretion. Similar studies performed in the nongoitrous area of the island showed that, in contrast, the ingestion of cassava grown in that area did not modify thyroid uptake to any appreciable extent (Table 2).

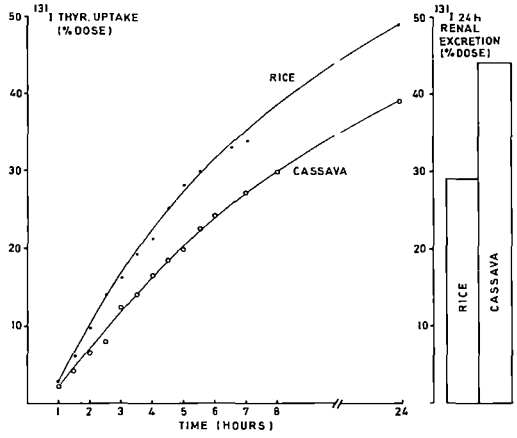


FIG. 1. Comparison of the thyroid uptake and urinary excretion of radioiodine in a Belgian subject after a meal of rice and after a meal of cassava grown in the goitrous area of Idjwi Island.

## Identification of Antithyroidal Substances

We identified the antithyroidal substance among the foods consumed in Idjwi. The foods most frequently suggested as containing goitrogenic factors belong to the family Cruciferae, *Brassica* species. These plants contain relatively large amounts of thioglucosides, the enzymatic hydrolysis of which releases SCN and allied substances (Van Etten 1969). Enzymatic hydrolysis occurs when the wet, unheated plant is crushed. Among the foods consumed in Idjwi, beans, peanuts, pumpkin seeds, cassava flour, and cassava roots were analyzed for their thioglucoside content but no trace could be detected. It was then necessary to know whether the above-mentioned SCN-like action after cassava ingestion was not related to the endogenous production of SCN. Indeed it is well established that cassava contains cyanogenic

TABLE 3. Cyanide content of cassava from the goitrous area of Idjwi Island.

| | Pulp (*mg/kg* ± SE) | Integument (*mg/kg* ± SE) |
|---|---|---|
| Flour | 30 ± 22 | 139 ± 145 |
| Variety 1 | 163 ± 11 | 227 ± 14 |
| Variety 2 | 180 ± 3 | 284 ± 27 |
| Variety 3 | 454 ± 27 | 446 ± 23 |
| Variety 4 | 698 ± 88 | 963 ± 112 |

glucosides whose enzymatic hydrolysis releases cyanide. This occurs when plant tissue is traumatized or by intestinal microflora.

After ingestion of cassava, cyanide is absorbed by the gastrointestinal tract and rapidly detoxicated into SCN by an enzymatic reaction involving sulfurtransferase, which is widely distributed in various organs (Montgomery 1969). Among the foods consumed in Idjwi Island, which were analyzed, only cassava contained cyanogenic glucosides. Table 3 shows that the cyanide content of cassava flour is very low (about 30 mg/kg) and frequently nonexistent. Furthermore, in cassava roots the amounts of cyanide are higher in the integument than in the pulp. The varieties of cassava roots analyzed are not identified botanically but they were distinguished by the inhabitants of the island because of their degree of toxicity. No significant difference in cyanide content was observed between cassava roots from the two regions of the island.

These results led us to study the antithyroid action of a single meal of cassava and to compare it with the action produced by acute administration of SCN in iodine-deprived rats. Four groups of rats were administered orally: distilled water, 1 mg SCN, 2 mg SCN, and 5 g cassava roots respectively and immediately injected intraperitoneally with $^{131}I$. In each group, rats were killed 2 h and 8 h after the injection. Table 4 shows that,

TABLE 4. Plasma thiocyanate concentration (*mg/100 ml*, ± SE) of rats after a single ingestion of different diets.

| Diet | Single ingestion | | No ingestion (*controls*) |
|---|---|---|---|
| | After 2 h | After 8 h | |
| Water | 0.51 ± 0.02 | 0.51 ± 0.02 | 0.54 ± 0.02 |
| SCN (1 mg) | 2.30 ± 0.15 | 1.27 ± 0.02 | 0.55 ± 0.02 |
| SCN (2 mg) | 4.89 ± 0.07 | 1.65 ± 0.07 | 0.58 ± 0.02 |
| Cassava | 1.51 ± 0.13 | 1.94 ± 0.08 | 0.73 ± 0.04 |

TABLE 5. Thyroid uptake of $^{131}I$ (*% of dose*) in rats after a single ingestion of different diets.

| Diet | After 2 h | After 8 h |
|---|---|---|
| Water | 25.3 ± 2.6 | 67.7 ± 3.1 |
| SCN (1 mg) | 23.1 ± 1.2 | 57.1 ± 7.0 |
| SCN (2 mg) | 14.1 ± 2.0 | 51.9 ± 2.6 |
| Cassava | 12.9 ± 6.6 | 40.1 ± 5.7 |

compared with rats administered distilled water, the plasma SCN concentration of rats fed cassava progressively increased. On the other hand, the administration of SCN rapidly increased the plasma SCN concentration to very high levels (2 h) which then decreased to reach at 8 h values lower than those observed in rats fed cassava. Table 5 shows that thyroid uptake of radioiodine was reduced by administration of cassava and SCN, and was proportional to the dose of SCN administered in the SCN groups (1 and 2 mg). After 8 h, the thyroid uptake of $^{131}I$ was lower in the cassava group than in the SCN groups. It is noteworthy that the thyroid uptake of radioiodine is closely related to the plasma SCN concentration; the uptake of $^{131}I$ is inversely proportional to the plasma SCN concentration. Table 6 shows that SCN excretion (8 h) was considerably increased by SCN administration proportionally to the dose of SCN ingested and only slightly increased by cassava ingestion. This is probably due to the progressive production of SCN in rats fed cassava when SCN is already eliminated in SCN-administered rats.

The results obtained in man and in animal are in in agreement with the view that the antithyroid action of cassava is related to the endogenous production of SCN from cyanide released by the cyanogenic glucosides present in cassava. Furthermore, the results suggest that this food in the presence of an iodine deficiency constitutes a goitrogenic factor, the existence of which was

TABLE 6. Thiocyanate excretion 8 h after ingestion of a single meal of different diets.

| Diet | SCN (*mg* ± SE) |
|---|---|
| Water | 0.009 ± 0.002 |
| SCN (1 mg) | 0.419 ± 0.028 |
| SCN (2 mg) | 1.253 ± 0.268 |
| Cassava | 0.056 ± 0.013 |

proposed by Delange and Ermans (1971), and that it can play a role in the etiology of endemic goiter in Idjwi island. However, the results of the food analysis do not allow us to suggest that cassava is the single factor responsible for the difference in goiter prevalence between the north and the south of Idjwi Island.

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# Mechanism of the Goitrogenic Action of Cassava[1,2]

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ERMANS, A. M., M. VAN DER VELDEN, J. KINTHAERT, AND F. DELANGE. 1973. Mechanism of the goitrogenic action of cassava, p. 153–157. *In* Chronic cassava toxicity: proceedings of an interdisciplinary workshop, London, England, 29–30 January 1973. Int. Develop. Res. Centre Monogr. IDRC-010e.

**Abstract** Long-term action of cassava tubers added to a Remington diet has been tested in rats and compared with the supplementation of graded doses of thiocyanate. Both cassava and thiocyanate induce firstly: depletion of the thyroidal iodine stores, major abnormalities of intrathyroidal metabolism, reduction of plasma PB $I^{127}$ and, secondly, moderate increase of plasma thiocyanate and a striking increase of plasma $^{35}SCN$ turnover. Thyroidal $^{131}I$ uptake is not inhibited at all. All findings show a qualitative and quantitative similarity between the effects of 10 g of cassava tubers and 1–2 mg of SCN.

It is concluded that a) the antithyroid action of cassava is caused by the endogenous production of SCN related to the conversion of cyanide, and b) in rats overloaded with large doses of SCN a renal adaptation mechanism is induced which strikingly reduces the plasma level of SCN.

**Résumé** Les auteurs ont mesuré sur des rats l'action à long terme de l'addition de tubercules de manioc au régime de Remington, et l'ont comparé aux effets d'un supplément dosé de thiocyanate. Le manioc, tout comme le thiocyanate, provoque en premier lieu: épuisement des réserves d'iode de la thyroïde, anomalies majeures du métabolisme intrathyroïdien, réduction du PB $I^{127}$ dans le plasma et, en second lieu, augmentation modérée du thiocyanate plasmatique et augmentation frappante du taux de renouvellement du $^{35}SCN$ plasmatique. La captation de $^{131}I$ par la thyroïde n'est aucunement paralysée. Les résultats s'accordent à démontrer des ressemblances qualitatives et quantatives entre les effets de 10 g de tubercules de manioc et 1 ou 2 mg de SCN.

Les auteurs en concluent que a) l'action antithyroïdienne du manioc est due à la production endogène de SCN associée à la conversion du cyanure, et b) la surcharge de fortes doses de SCN met en branle, chez le rat, un mécanisme d'adaptation rénale qui réduit de façon frappante le niveau de SCN dans le plasma.

CLINICAL and experimental data reported in Delange et al. (1973) suggest that the antithyroid activity of cassava could be related to the conversion of its cyanide content into thiocyanate (SCN) after consumption by humans. However, such an hypothesis seems difficult to accept according to some observations recorded in individuals living in endemic goiter areas as well with current concepts concerning the metabolism of SCN.

Firstly, administration of a single dose of SCN in rats induces a marked increase of its concentration in plasma; concomitantly, iodide uptake in the thyroid gland is partially or completely

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blocked (Wollman 1962). Secondly, SCN ion is excreted very slowly in urine and its catabolic rate is very low (Sollman 1957). Prolonged ingestion of foodstuffs containing SCN precursors would be therefore expected to induce long-term increases of plasma SCN levels, and subsequently a persistent reduction of the iodide uptake in the thyroid gland should be observed. This view disagrees with the elevated values of $^{131}I$ uptake found in all severe goiter endemics and particularly in the Idjwi Island (Delange et al. 1968) in which one of the authors (F.D.) suggested that cassava could play a determining role in the development of the disease (Delange and Ermans 1971).

Few experimental data are available on the long-term influence of continuous administration of SCN or its precursors. The purpose of the present study was to reevaluate the modifications of SCN and iodine metabolism in rats submitted over a long period to a diet containing either cassava or graded doses of SCN (Ermans et al. 1972).

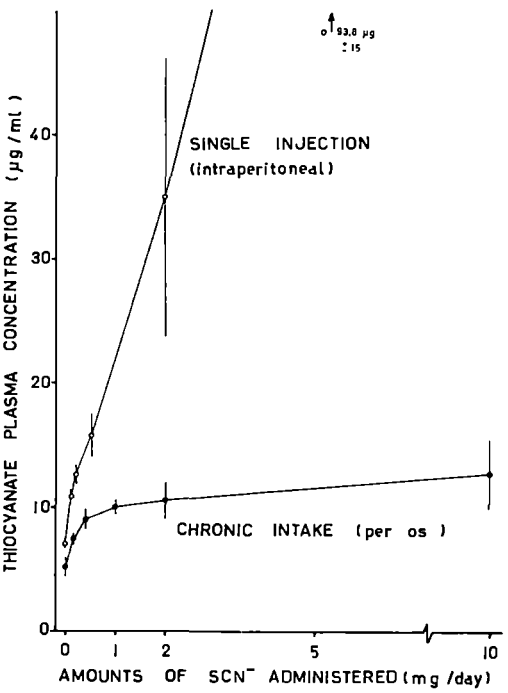


FIG. 1. Thiocyanate levels in serum of rats fed a Remington diet after administration of SCN doses ranging from 0.1 to 5.0 mg. A single intraperitoneal dose of SCN is administered in "acute experiments"; in "chronic experiments," the same dose is mixed every day with the Remington diet for 4 weeks (from Ermans et al. 1972).

## Materials and Methods

The basic experimental protocol consisted in estimating various parameters of iodine and thiocyanate metabolism in 250 male White Star rats subjected to a low-iodine diet (Remington), supplemented with varying doses of thiocyanates ranging from 0.1 to 10 mg/day over a period of 4–6 weeks. The same estimations were made in rats subjected to a similar diet with the addition of 10 g of fresh cassava roots per day. Control experiments were carried out after a single intraperitoneal injection of the same doses of SCN. Some investigations were conducted after continuous supplementation with 10 µg of iodide per day.

At the end of the administration of the various diets, the following parameters were estimated for SCN distribution: plasma SCN concentration; urinary SCN excretion; SCN renal clearance; and disposal rate of plasma $^{35}S$-labelled SCN. Also estimated were the parameters for iodine metabolism: thyroid $^{131}I$ uptake (4 h); iodide thyroid clearance; iodide renal clearance; iodine thyroid content; plasma PB $^{127}I$; and pattern of thyroid hormone synthesis.

## Results

### Modifications of SCN Distribution Induced by Overload

Figure 1 shows a striking difference between the plasma levels of SCN under acute and chronic conditions. Four hours after a single intraperitoneal injection of SCN, with doses ranging from 1 to 5 mg, a sharp increase of SCN plasma levels takes place, whereas only a moderate increase of this level, to about 10 µg/ml, is observed (regardless of dosage) when thiocyanate is administered orally every day for several weeks. These samples were collected in the post-absorption period (Table 1); moderately higher values were observed during the feeding period (Table 2).

In investigating chronic effects (Table 1), almost the entire supplement of SCN (85%) is recovered in the urine. Renal clearances of SCN (Table 2) as well as the plasma disposal rate of $^{35}S$-labelled thiocyanate (Table 1) are strikingly increased;

TABLE 1. Modifications of iodine and thiocyanate metabolism induced by prolonged administration of cassava or graded doses of thiocyanate. (Mean calculated on the basis of 5–9 individual values. Value statistically different from the control value: $*P < 0.01$; $**P < 0.001$.)

| | | SCN supplement (*mg/day*) | | | | Cassava (*10 g/day*) |
|---|---|---|---|---|---|---|
| | Controls | 1 | 2 | 5 | 10 | |
| Thyroid I content[a] (*μg*) | 1.0 | 0.7 | 0.6* | – | – | 0.5** |
| Thyroid I content[b] (*μg*) | 11.9 | 10.5 | 7.9** | 7.6** | – | 9.2** |
| $^{131}$I thyroid uptake[a,b] (*4 h, %/dose*) | 8.5 | 11.2** | 10.2 | 13.3** | – | 9.7 |
| Plasma PB$^{127}$I | 1.8 | 1.3** | 1.2** | – | – | 1.1** |
| $^{125}$I-labelled iodoamino acids in thyroid glands | | | | | | |
| MIT:DIT | 0.89 | 1.31** | 1.62** | – | 2.00** | 1.63** |
| as T4 (%) | 21.0 | 18.0 | 11.0** | – | 6.0** | 13.0** |
| as T3 (%) | 9.0 | 10.0 | 11.0 | – | 13.0 | 9.0 |
| $^{35}$SCN plasma disposal rate (*fraction/h*) | 0.01 | 0.05 | 0.14 | 0.22 | – | 0.13 |

[a]Estimates carried out during post-absorption period.
[b]Diets supplemented with iodide (10 *μg* day).

TABLE 2. Kinetics of iodide and thiocyanate after prolonged administration of SCN. Investigations were carried out during the feeding period.

| | | SCN supplement/day | |
|---|---|---|---|
| | Controls | 1 mg | 5 mg |
| SCN plasma concn (*μg*/ml) | 5.3 | 10.6** | 22.8** |
| Renal clearance of SCN ($10^{-2}$ ml/min) | 0.44 | 4.20** | 15.03** |
| Renal clearance of iodide ($10^{-2}$ ml/min) | 6.7 | 8.2 | 9.3** |
| Thyroidal clearance of iodide ($10^{-2}$ ml/min) | 1.5 | 1.4 | 1.2** |
| Thyroid $^{131}$I uptake (%/day, 4 h) | 5.4 | 5.6 | 4.3 |

modifications of both parameters are found to be strictly related to the size of SCN intake.

### Modifications of Iodine Metabolism Induced by SCN Overload

No inhibitory effect of the $^{131}$I thyroid uptakes was evidenced during the post-absorption period in chronic toxicity experiments. For most of the SCN doses used, $^{131}$I uptake was significantly increased (Table 1). A transient partial inhibition was, however, observed during the feeding period in the 5-mg group. In the same animals, the renal clearance of iodide was significantly increased (Table 2).

Iodine content of the thyroid glands was markedly decreased after chronic overload with large doses of SCN; the extent of the iodine depletion is proportional to the dose given and appears definitely more severe in iodine-deficient rats (Table 1). In the latter condition, marked abnormalities of the distribution of $^{125}$I-labelled iodoamino acids were observed in the thyroid glands (Table 1), i.e. an increase of the monoiodotyrosine: diiodotyrosine (MIT:DIT) ratio and a decrease of thyroxine (T4) content; on the contrary, triiodotyrosine (T3) content remains unmodified in the same experimental conditions. The extent of the modifications of the MIT:DIT ratio and of the T4 content was also found depending quantitatively on the size of the SCN dose. Chronic SCN overload also induces a marked reduction of the plasma protein-bound iodine (PB$^{127}$I).

### Modifications Induced by Chronic Feeding with Cassava

Chronic administration of cassava to rats significantly increases SCN concentration as well as the $^{35}$S-labelled SCN disposal rate, in plasma. The value of the disposal rate (0.13/h) coincides with that obtained for the rats (Table 1) fed 2 mg SCN daily (0.12/h).

Modifications of the iodine metabolism were iodine depletion of the thyroid glands with increased MIT:DIT ratio and decreased T4 content and at least decrease of the plasma PB$^{127}$I (Table 1). No inhibitory effect of $^{131}$I thyroid uptake was evidenced.

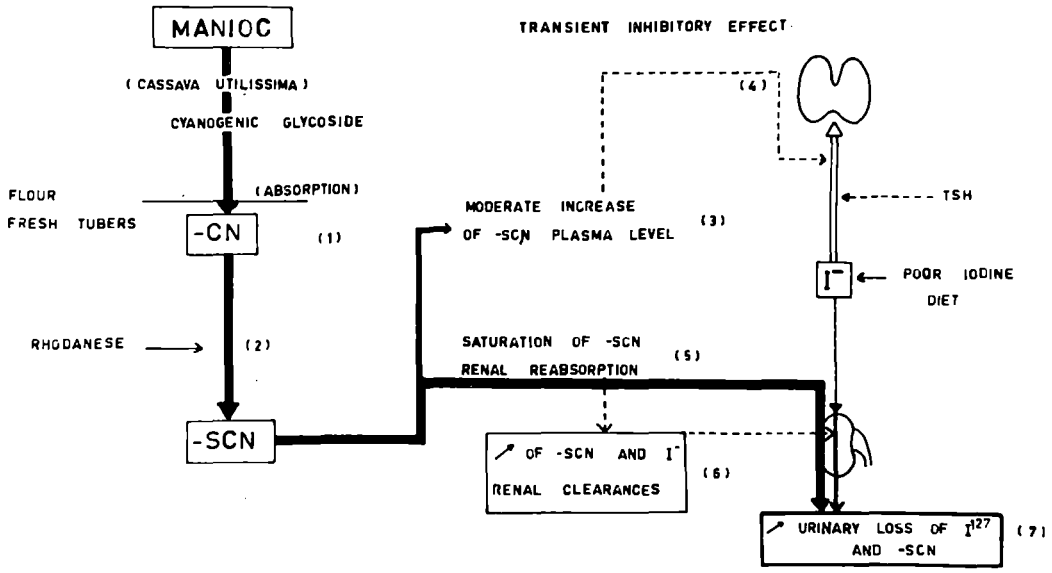


FIG. 2. Tentative model of the mechanism of the goitrogenic action of cassava in iodine-deficient rats. The major step lies in the depletion of the thyroid iodine stores by a real loss of iodide.

Each of these modifications also correspond quantitatively to those observed in rats treated with 1–2 mg SCN daily.

## Discussion and Conclusions

Complete similarity of the effects induced by giving either cassava or definite amounts of SCN confirm the hypothesis that the antithyroid activity of cassava is related to the endogenous production of SCN. This similarity was indeed checked qualitatively as well as quantitatively for the modifications of the SCN and the iodine metabolism.

The main effect of the prolonged consumption of cassava is a marked depletion of the iodine stores; the degree of this depletion appears strikingly severe in the absence of iodine supplementation. It is interesting to point out that the abnormalities of hormone synthesis are definitely those found in very poorly iodinated thyroglobulin (Ermans et al. 1968) or after long-term administration of low-iodine diets (Studer and Greer 1965).

Present observations moreover demonstrate that long-term consumption of cassava, as well as graded doses of SCN, induces a marked increase of the excretion rate of SCN, with the main consequence being a striking acceleration of its disposal rate from plasma. This adaptation mechanism accounts for the marked difference noticed between acute and chronic administration; it also gives an adequate explanation for the absence of inhibition of the $^{131}I$ thyroid uptake in the post-absorption period after chronic administration of these diets.

Two mechanisms can be advocated to explain the iodine depletion: on the one hand a transient block of iodide accumulation in the thyroid gland during the feeding period (Delange et al. 1973), and on the other hand an increase of the renal clearance of iodide. The relative importance of both factors could not be estimated on the basis of present investigations.

It seems of particular interest to mention that all abnormalities induced on the thyroid function by cassava consumption could not be distinguished qualitatively from those related to an insufficient iodine intake, with the exception of the observations specifically related to the SCN metabolism.

In conclusion, prolonged administration of cassava (or of SCN) in iodine-deficient rats provides a satisfactory model (Fig. 2) to assess the role of this foodstuff in the pathogenesis of endemic goiter.

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# Summary of the General Discussion

● Screening for acyanogenesis and low cyanide levels in the extensive collections of cultivated lines of cassava which are available and also of uncultivated "wild" material would be desirable. No acyanogenic strains of *M. esculenta* are known (although one was reported in Indo-China some years ago). The most promising material might be expected to be at the centre of origin of *M. esculenta* (now believed to be in North Colombia) and also at the periphery of its natural distribution. It is unlikely that an acyanogenic line, once developed, would revert to cyanogenesis (on the basis of experience over 20 years with acyanogenic white clover lines).

● The intermediates in the biosynthesis of linamarin and lotaustralin do not accumulate in the plant and do not represent a toxicological hazard. If acyanogenic cassava plants were discovered, it is likely, on the basis of experience with other species, that the metabolic block would be at the commencement of the pathway as a result of mutation of a regulatory gene which eliminates the entire pathway. The possibility of inhibition of the biosynthesis by the use on the cassava crop of an inhibitor selective for the cyanoglucoside pathway should be considered, although no suitable substances can be suggested at this stage.

● Knowledge of the physiology of the cassava plant insofar as it affects cyanoglucoside levels requires further investigation under conditions of controlled environment and controlled mineral nutrition. Such information may well be obtainable as an adjunct to experiments aimed at studying other parameters such as photosynthetic efficiency. It is particularly important to establish whether or not the primary site of cyanoglucoside synthesis is the leaves, followed by cyanoglucoside translocation to the roots. The extent to which the glucosides are synthesized in the roots can be tested by $C^{14}$-valine feeding experiments using cores of root tissue. The cyanoglucoside content of stem phloem sap should also be determined if possible.

● If the leaf proves to be the site of cyanogenesis it will be possible to screen for cyanogen content using leaf tissue in which case zero linamarin rather than zero linamarase should be sought. As the gene(s) controlling cyanogenesis may be recessive, it would be preferable for inbred lines to be used for the screening process.

● The techniques of radiation- or mutation-breeding merit attention; although frequently unsuccessful as a breeding tool, it should be borne in mind that, in the case of acyanogenesis in cassava, it is desired to eliminate a character rather than to develop a new one and hence the chances of success may well be greater than usual.

● The advantages of varieties with low- or zero-cyanide contents in the interior of the tuber, combined with high cyanide levels in the skin were discussed, from the viewpoint of possible pest resistance. In Thailand, cassava with high cyanide levels is grown with low cyanide varieties, but it seems likely that these mixed crops of bitter and sweet varieties are grown essentially to reduce pilfering by humans.

● However, there is evidence that, under some circumstances, cyanoglucosides can have a protective role in plants and therefore it is possible that an acyanogenic variety of cassava could be more susceptible to pathogens and to pests than the existing cyanogenic form. Conflicting comments on the effects of breeding-out of alkaloids and of thioglucosides from other species only strengthened the view that no prediction could be made for cassava about possible susceptibility. Subjective observations on the occurrence of dead partridges and other animals near cassava crops were mentioned, while grasshoppers had also been seen eating some varieties of cassava, but not others. It was not known whether this differential eating was associated with the cyanogenic glucoside content of the leaves.

● The difficulties encountered over the apparent retention of cyanide in prepared cassava were discussed. Cyanhydrins, as a class, have a wide range of dissociation constants with the equilibrium in tissues well over towards cyanhydrin. The concentration of hexose sugars is high (about 4%) in the tuberous roots of cassava and hence interaction between the HCN released and these sugars is highly likely. Thus the HCN could be trapped as cyanhydrin, being released when water or acid is added. It was stated that cassava which has been fried in cooking oils retains the capacity to release HCN whether or not linamarase is added later. A possible explanation is that the HCN produced before the $\beta$-glucosidase is destroyed by heat reacts with carbonyl groups in these oils.

● Apart from the nutritional problems implied, retention of HCN in processed cassava negates any consistent method of estimating the cyanogenic glucoside content of tuberous roots by measuring the CN produced on hydrolysis. Clearly, this also casts doubt upon the validity of estimations of cyanogenic glucosides in other species. Indeed, when known quantities of substrate were added with linamarase to cyanogenic plant tissue, only 50% of the added part could be recovered. Although it was possible that an enzyme inhibitor was present, it was more likely that the HCN released was trapped by aldehydes and sugars.

● Although the discussion concentrated on HCN, the possibility that cyanogenic glucosides themselves could be trapped was considered as a possibility. Reference was also made to the fact that about half of the total nitrogen of cassava is non-protein nitrogen and it was suggested that nitriles might possibly play a role in cassava toxicity.

● Indeed, the possibility that substances other than cyanogenic glucosides might be responsible for the clinical conditions described in humans was discussed. However, experiments in New Zealand with rats and guinea pigs, using the cyanogenic or acyanogenic forms of white clover, produced thyroid changes in complete agreement with those recounted at the workshop by the Belgian team. The essential difference between the two experimental conditions was the control feed; acyanogenic white clover as opposed to a Remington diet. Thus other plant constituents are probably unimportant, because it is likely that cyanogenic and acyanogenic white clover plants of the same commercial variety differ only in the cyanogenic glucoside content.

● Participants questioned whether linamarin itself is toxic and noted that both linamarin and lotaustralin are relatively stable compounds which are unchanged in 0.1 N acid over 39°C during 48 h, i.e. under the conditions of acidity encountered in the stomach, they are also unchanged by boiling in water at 100°C. It was suggested that while linamarin could be absorbed through the small intestine, it would otherwise be degraded in the large intestine particularly by enzymes produced by the gut flora. Certainly HCN is present in fresh faeces, while cyanogenesis in sewage can present a health hazard. It was concluded that little was known about the digestion of linamarin on the one hand, and the possible variation in gut flora, related to diet, on the other.

● However, in feeding trials, it seems reasonable to distinguish between monogastric and polygastric mammals. The stomachs of ruminants were like fermentation tanks, in which breakdown of cyanogenic substances was probable. Ruminants have a higher tolerance to HCN than the other mammals tested. There is ample evidence to show that high levels of cassava can be fed to pigs and poultry with no ill effects, providing the diet is balanced and possibly enriched with methionine. Instances were cited of dried cassava being used at up to 69% in compounded feeds, and of fresh cassava as the sole carbohydrate source. Earlier reports of toxic effects in livestock were probably associated with unbalanced diets.

● In some parts of Africa, two important human diseases, tropical ataxic neuropathy and endemic goitre, appear to be associated with a high cassava intake. Ataxic neuropathy is a disease of poor people who eat cassava, and little else, twice daily. Under such circumstances, the disease may have an incidence many times that of some well-known diseases of affluent society. In Nigeria, ataxic neuropathy is always associated with an unbalanced diet, especially one low in meat which may have some protective effect associated with its high content of sulfur-containing amino acids. However, in one area where both cassava and fish are consumed in large quantities, the incidence of neuropathy is high.

● In Zaïre, goitre rather than neuropathy has been reported, and this condition appears to be associated with the formation of thiocyanate (SCN) from cyanide detoxification. However, the evidence for this is purely circumstantial based on feeding experiments and requires verification before SCN can be definitely incriminated as the sole causative factor. In any event, a low iodine intake and nutritional status appear to be preconditions for disturbances in iodine metabolism associated with high cassava intakes. In some cassava-eating patients with goitre, the symptoms have disappeared following the injection of iodized oil.

● Apart from its association with goitre, high cassava intake may also be associated with congenital cretinism of the newborn especially when the iodine and nutritional status of the mother in the last trimester of pregnancy are low. In discussing this, it was noted that cows eating cruciferous plants containing thioglucosides do not pass iodine on to their calves.

● There appeared to be a considerable lack of organized information on traditional methods of detoxicating or otherwise preparing cassava for human food and it was suggested that there was a need to establish internationally acceptable standards for permissible levels of linamarin and of free or available cyanide, as has been done for other glycoside-containing foodstuffs. In view of the current concern with toxic factors in food and the good market potential that seems to exist for cassava products, this subject would seem to warrant further study.

● It was recognized that the prospects for reducing toxicity by changing rural processing procedures were limited, due to the strength of traditional customs. Nevertheless cassava is a crop that has relatively recently been introduced to many countries suggesting that new technology and practices can be introduced if suitable new (and perhaps fortified) processed cassava products can be developed. The need for such products is apparent from the levels of cyanide in some widely used cassava products—e.g. purupuru in West Africa may be consumed in quantities which give a daily cyanide intake of 20–50 mg. In some purupuru-eating areas, ataxic neuropathy assumes endemic importance. Likewise in Idjwi Island where the level of goitre is high, the population eat both cassava flour which is low in cyanide and raw cassava which has a very high cyanide level.

- For further experimental work on toxicity, it may be desirable to use species other than rats and pigs. The major problem in this type of work concerns the comparability of the physiological age of the animals used and this makes it difficult and perhaps misleading to extrapolate to man. It also appears to be difficult to reproduce neurological diseases in animals. Rats seem to be very resistant to cyanide and show an astonishing capacity for renal clearance of orally administered cyanide. The need for primate work was stressed. However, it is important not to underestimate the difficulties of using primates.

- For further animal work on both toxicology and metabolism, a substantial amount of purified linamarin would be desirable. Smaller quantities of isotopically labelled linamarin are also needed for work on animal physiology and biochemistry. For such work, it should be possible to synthesize linamarin or to extract it from suitable plant material. It was suggested that this subject should be investigated from the standpoint of obtaining a large enough supply for a cooperative interdisciplinary research program on cassava toxicity.

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